EFFECT OF METHADONE ON PLASMA AND CEREBROSPINAL FLUID CALCITONIN LEVELS AFTER DAILY REPEATED METHADONE ADMINISTRATION TO SHEEP

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In previous investigations we observed that daily repeated administration of cocaine, nicotine and phenobarbital resulted in increase of the plasma and cerebrospinal fluid (CSF) concentrations in sheep. In recent years methadon is used in the maintenance treatment of narcotic adicts. In the present study we investigated the effect of methadone on the plasma and CSF calcitonin levels after daily repeated administration of methadone to sheep.

The effect of methadone was studied in 3 male sheep. Before the experiments catheters were inserted under anaesthesia into the subarachnoid space and into the arteria cervicalis superficialis. On the third postoperative day after basal blood and CSF samples (controls) were obtained, methadone-HCL was i.a. administered daily up to 3 weeks. The dosis administered were: days 1 and 2 = 5 mg/day, day 3 = 7.5 mg/day, and from day 4 = 10 mg/day. Blood and CSF samples were obtained simultaneously at 1 hs intervals up to 10 hs after methadone administration.

The CT (1-32 amino acids) concentrations in plasma and CSF were measured by RIA (IRE, Belgium). In addition also total calcium (Ca) levels were determined by complexometric titration.

The results showed that methadone administration is followed by decrease of the CSF CT concentrations, immediately in the first day of administration. The levels decreased from 60 pg/ml up to 40 pg/ml approximately. The plasma CT values decreased likewise, but also from the 5th days after beginning of the experiment. The concentrations declined from 90 pg/ml up to 25 pg/ml approximately. The Ca levels remained unchanged.

The therapeutic effect of methadone used in the maintenance treatment of narcotic addicts, based on the one hand, on the methadone ability to blocked the morphine receptors. Consequently, the heroine dependence and require are not more present. On the other hand, methadone is not inebriate. The patients are able to work and this is again the first step in change of life stile and resocialisation.

In recent years the presence of CT and its binding sites, as well as secretion and degradation of the hormone in the central nervous system (CNS) have been demonstrated. CT effects on the CNS were also repeated observed. It is supposed that the hormone may be neurotransmitter/neuromodulator.

Early studies have shown plasma CT levels in addicts to be significantly higher than these in control. As previously mentioned, we observed that administration of cocaine, nicotine and phenobarbital resulted in increase of the CT plasma and CSF levels in sheep. The mechanism of this effect can be related to dopamine systeme alteration under narcotic.

Based on our present results, we suppose that CT participate, at least in part, in the methadone effect on addicts.

PHENOBARBITAL KINETICS IN PLASMA AND CEREBROSPINAL FLUID AFTER DAILY REPEATED ADMINISTRATION OF PHENOBARBITAL TO SHEEP

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In this study we investigated the kinetic of phenobarbital in sheep plasma and cerebrospinal fluid (CSF) after daily repeated administration. The study was proved in three male sheep. Before the experiments catheters were inserted under anaesthesia into the subarachnoidal space, into the arteria cervicalis superficialis and into the vein (scapula region). On the third postoperative day, after basal blood and CSF samples (controls) were obtained, 0.1 g phenobarbital was daily, up to 28 days, i.v. administered. Blood samples from the artery and CSF were obtained at 1 hs intervals up to 10 hs after phenobarbital administration. Daily urine samples (24 hs urin) were also obtained. In addition after killing of the sheep, the phenobarbital concentrations in liver, bladder, bile, brain, kidney, pancreas, thymus, lung, heart muscle, stomach wall, spleen, adrenal and fatty tissue were measured. The phenobarbital concentrations in the samples were determined by radioimmunoassay, coat a count antibodies coated polypropylene tubes (Biermann, FRG). The tracer was 125-I-labelled barbiturate. The lower detection limit was 6.2 ng/ml. The antibody used react with phenobarbital but also with its metabolites. Consequently, our results present the sum of phenobarbital and its metabolites.

The results showed the highest plasma phenobarbital concentration in the first 60 min after administration (5 ug/ml approximately). The levels declined continuous, however in the 4th and 6th hs increase of the phenobarbital concentrations was again observed. The highest CSF phenobarbital values were found likewise in the first 60 min (3 ug/ml). The concentrations decline then continuous. A second or third increase as in plasma, was not observed. The urine phenobarbital concentrations in all three sheep were similar approximately (mean values: 12.6 ug/ml; 10.3 ug/ml and 11.2 ug/ml). In the organs the highest values were found in: bile, kidney, liver, bladder (12.2 ug/g; 6 ug/g; 3,4 ug/g and 2.7 ug/g respectively). In the remained organs the concentrations ranged from 0.2 up to 1.0 ug/g. In brain 1.1 ug/g approximately were found. Correlation between the phenobarbital concentrations in the different samples was not present.

The in this study observed increase of the phenobarbital levels in blood in the 4th and 6th hours respectively after drug administration, indicated the existence of an enterohepatic circulation. However, we can not exclude that the increase is only result of phenobarbital release from tissues.

Moreover, the concentrations measured into the CSF, showed that phenobarbital is immediately transfered from blood into the CSF. The phenobarbital is transfered also from CSF into the brain tissues. The relative high concentrations found in CSF and also brain, indicated that the blood-CSF-, and CSF-brain-barriers are permeable for phenobarbital.

EXCRETION BALANCE AND URINARY METABOLISM OF ^{3}H-FCE 22178 IN RAT, DOG AND MAN.

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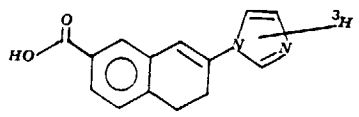
FCE 22178, 5,6-dihydro-7-(1H-imidazol-1-yl)-2-naphtalene carboxylic acid, is a new derivative which showed strong, long-lasting inhibition of thromboxane (TxA_2) synthase and appreciable stimulation of Prostacyclin (PGI_2) (1).

^{3}H-FCE 22178 (Figure 1) was orally administered to rats and dogs at a dose of 10 mg/kg and to man at a dose of 400 mg.

Total radioactivity was measured in urine and faeces up to 96 h. In all species most radioactivity was excreted within 24 h and only small percentages were eliminated over the following days. In man radioactivity was eliminated almost exclusively in urine; in the rat urine was a major elimination route, whereas in the dog the urinary and faecal elimination routes were equally important. Radioactive recovery, expressed as mean percent of the administered dose ± SEM, n = x, was as follows:

Species	Rat		Dog		Man	
n	4		3		4	
	Urine	Faeces	Urine	Faeces	Urine	Faeces
0-24	61.79±2.89	25.20±3.74	41.13±1.46	44.79±2.31	80.50±6.60	5.40(n=2)
0-96	64.61±3.09	27.53±3.36	43.88±1.73	49.09±0.80	92.03±8.39	8.60±5.44

In the 0-24 h rat urine, free unchanged FCE 22178 accounted for 41% of the administered dose, whereas the conjugated form accounted for 19%. The percentages of free and conjugated FCE 22178 in the 0-24 h dog urine were 26% and 15% of the dose, respectively. In contrast with the rat and the dog, in which the drug was excreted mostly in the free form, in man conjugation seems to play a major role in the metabolism of FCE 22178. In fact after oral administration of 400 mg of FCE 22178 to healthy volunteers the drug was recovered in urine mostly in the conjugated form (2).



(1) Chiari et al. - Joint Meeting of Medicinal Chemistry, Rimini (Italy), May 21-25, 1985.
(2) Bertin et al. - Congresso Nazionale di Farmacocinetica: Aspetti Teorici e Pratici, Siena (Italy), May 27-30, 1990.

STABILIZATION OF DRUG METABOLIZING ENZYMES IN HEPATIC CULTURED CELLS

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Several in vitro models have been recently developed in order to study drug biotransformation or expression and regulation of liver genes. Hepatocytes in primary culture represent the first and main used model. Improvements of the culture conditions (extracellular matrix, adapted media, co-cultures with epithelial cells) allowed to partially circumvent dedifferenciation. We demonstrated that the method used to isolate hepatocytes played an important role in stabilizing hepatic functions during culture. The use of EDTA instead of collagenase as the dissociating agents resulted in more stable hepatic functions and drug metabolizing enzymes (phase I and II-reactions).

The other approach to obtain in vitro hepatic models with a differentiated phenotype is the culture of established cell lines. We compared the expression of different cytochrome P-450 isoenzymes and UDP-glucuronosyltransferases in rat hepatoma cell lines and immortalized hepatocytes with freshly isolated and cultured hepatocytes. Phase I enzymes were expressed at a higher level in immortalized hepatocytes.

PREDICTION OF INTRINSIC CLEARANCE OF LOXTIDINE FROM KINETIC STUDIES IN RAT, DOG AND HUMAN HEPATOCYTES.

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Pharmacokinetic parameters of loxtidine, a potent long acting histamine H_2-receptor antagonist, were determined in rat, dog and man after intravenous administration.

Loxtidine has a low hepatic extraction ratio in rat, dog and man. Intrinsic clearance values of approximately 29, 10 and 1ml/minute/kg were observed in rat, dog and man respectively. Kinetic studies with loxtidine in isolated rat, dog and human hepatocytes were used to calculate K_m and V_{max} values from which intrinsic clearance values in vitro were derived. The values obtained were in the order rat > dog > man and a good correlation was obtained with the corresponding in vivo values.

BIOTRANSFORMATION OF BAY R 3783 IN RATS AND DOGS

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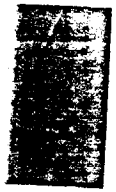
The biotransformation of BAY r 3783, 3-(4-chlorophenyl)-2,2-dimethyl-3-hydroxy-4-(1,2,4-triazol-1-yl)-butanal-oxim-O-methylether, a new triazole antifungal, has been studied in vivo and in vitro in rats and dogs.

In male rats, BAY r 3783 is metabolized almost completely. Independent of the route of administration, unchanged drug represents less than 0.6% of the dose in the excreta.
Following oral as well as intravenous administration of [triazole-3,5-^{14}C]BAY r 3783, more than 20 metabolites are detected in rat urine.
After intraduodenal application of [^{14}C]BAY r 3783 to bile duct-cannulated rats, on average more than 90% of the dose are eliminated via the bile. The metabolic profiles in rat bile and urine are very different, with the glucuronide of the demethylated drug (metabolite M3) predominating in bile and 1,2,4-triazole predominating in urine.

In dogs, BAY r 3783 is metabolized completely.
After administration of [^{14}C]BAY r 3783 the radioactivity is mainly excreted renally by intact as well as by bile duct-cannulated dogs. In dog urine, 1,2,4-triazole is the main biotransformation product, whereas in bile two main metabolites (M3 and M26) are detected. Formation of metabolite M26, i.e. hydroxylation of one methyl group and subsequent glucuronidation, indicates that biotransformation of BAY r 3783 in dogs follows two equally important pathways.

Following intraduodenal administration, the metabolites identified both in rat bile as well as in dog urine and bile represent about 70% of the dose.



P 4.10

METABOLISM OF CLEBOPRIDE IN ANIMALS AND MAN

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Clebopride (I) is a drug with antiemetic and prokinetic properties. The metabolism is in part known from earlier investigations. Within this study the metabolism of I in rat, dog and man was reinvestigated after administration of ^{14}C-clebopride labelled in two biochemically stable positions. HPLC with radioactivity detection, MS, NMR and IR spectroscopy were employed for isolation, structure elucidation and identification of two known and three new products of in vivo biotransformation. HPLC measurements allow a quantitative inter-species comparison of biliary and renal excretion of metabolites and parent compound. Different oxidative metabolic steps are involved like N-dealkylation, α-oxidation, aromatic hydroxylation, O-demethylation and N-oxidation. A newly detected O-glucuronide resulted from phase-II reaction.

P 4.11

METABOLISM OF A NEW CARDIOTONIC AGENT IN LABORATORY ANIMALS AND MAN

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RG W-2938 (I), 3,4-dihydro-3-methyl-6-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2(1H)-quinazolinone, is a new positive inotropic agent with vasodilatory properties. Following oral administration, biotransformation of (I) was studied by means of ^{14}C-labeled material, HPLC and tandem HPLC-TSP/MS techniques. Total radioactivity excreted in urine of rat, dog and man accounted for 48%, 40% and 92% of the dose. In all three species >96% of the urinary radioactivity was excreted within 24 hours and the radioactive profiles were qualitatively similar. Urinary elimination of (I) in the rat, dog and man accounted for 12%, 7% and 36% of the dose, respectively. The major urinary metabolite in the rat and dog was identified as a dehydrogenated-(I) (RG 12408) accounting for 12% and 8% of the dose, respectively. The major urinary metabolites in man (18% of the dose) have not yet been identified. In man, RG 12408 accounted for 6% of the dose excreted in urine. Low levels of demethylated-(I) (RG W-2937) have been detected in the urine of dogs and man. In a 26 week oral drug safety study in the albino rat, crystals were observed microscopically in urine sediments and the renal pelvis of high dose animals. Crystals isolated from the urine and kidney tissues of high dose rats were characterized. These samples were stored in formalin and as a result the parent compound and metabolites reacted with the formaldehyde in the solution. The new compounds had a longer HPLC retention times, but still gave the same mass spectra as the reference standards. The new compounds could be generated by mixing the synthetic standards with formalin. Samples from both urine and kidney were identified as containing (I) and RG 12408 as the major components. RG W-2937 and a dehydrogenated RG W-2937 were also present as minor components.

The metabolism and disposition of LY170680 - a novel leukotriene D4 antagonist.

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Compound LY170680 is a potent LTD4 antagonist currently undergoing clinical evaluation for the treatment of asthma. The metabolic fate of LY170680 has been studied in the rat. Oral bioavailability was 20%, with an elimination half-life of 4 hours. LY170680 was rapidly eliminated by first-pass metabolism and excreted via the bile. Low plasma concentrations of compound LY170680 after oral administration accounted for the lack of oral activity in the guinea-pig. Major metabolites of LY170680 have been identified as the products of omega and three successive beta oxidations of the hydrocarbon chain of the parent molecule. After administration of radiolabelled LY170680 by either the oral or intravenous routes, the compound and its metabolites were excreted in the bile with only 1-2% of the dose accounted for by radiocarbon in the urine. Compound LY170680 is intended for administration by inhalation in man. The fate of radiolabelled LY170680 administered as an inhalation aerosol has been studied in the rat. Pharmacologically active doses of LY170680 were administered to rats by inhalation. This route of administration was associated with a half-life of radiocarbon in the lung of 1.5 h.

THE ENZYMOLOGY OF RANITIDINE METABOLISM IN ISOLATED HEPATOCYTES FROM DOG

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N-Oxidation of ranitidine was inhibited by 5-500μM methimazole (MMI) (41-96%) and thiourea (ThU) (27-97%) in a manner unaffected by addition of 1mM reduced glutathione (GSH). N-Benzylimidazole (BZI) (≤2.5mM) or SKF 525-A (≤250μM) did not inhibit N-oxidation. S-Oxidation was inhibited by MMI (7-22%) and ThU (0-20%) but not by ThU in the presence of GSH or by ≥200μM MMI with GSH. At 500μM MMI, inhibition was unaltered by GSH. Inhibition by BZI was 49-55% and by SKF 525-A 25-50%. N-Demethylation was inhibited by MMI (17-54%) and ThU (4-43%). Inclusion of GSH rescinded inhibition at and below 50μM MMI but did not alter the effects of 500μM MMI. The role of GSH in preventing ThU-mediated inhibition of demethylation could not be clearly discerned. However, inhibition of demethylation by BZI (12-45%) and SKF 525-A (6-26%) was observed. In the absence of inhibitors, GSH did not affect ranitidine metabolism. The results indicate that N-oxidation of ranitidine occurs via the flavin-containing monooxygenase (FMO). The ability of GSH to protect against MMI and ThU mediated inhibition contraindicates direct FMO involvement in S-oxidation and demethylation. Therefore S-oxidation and N-demethylation appear to occur via cytochrome P-450 mediated mechanisms variously susceptible to oxidation products of MMI and ThU.

NEW ASPECTS OF THE BIOTRANSFORMATION OF THE FUNGICIDE VINCLOZOLIN IN THE RAT AND OTHER SPECIES

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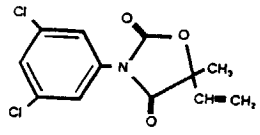
The biotransformation of vinclozolin (1), [3-(3,5 dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidin -2,4-dione], the active ingredient of the fungicide Ronilan® has been investigated in the rat, goat and hen. Rats were administered a single oral dose of ^{14}C-vinclozolin at 100 mg/kg. Hens and goats were administered 20 and 400 mg of ^{14}C-vinclozolin, respectively, daily for 5 days. The metabolite profiles in rat and goat urine, goat milk, hens' eggs and the livers of all three species were investigated.

Compound (3), previously identified as the major phase I metabolite of vinclozolin in the rat, was also found to be a major metabolite in goat and hen. In rat and goat, this metabolite was mainly excreted in urine as a glucuronide conjugate which was identified by mass spectrometry. Compound (3) is the product of extensive biotransformation of vinclozolin, involving hydrolytic cleavage of the heterocyclic ring and epoxidation of the vinyl group followed by hydration of the epoxide intermediate. No epoxide metabolites were identified, however the novel dihydroxylated metabolite (2), a possible intermediate in the formation of (3) was identified in rat urine and goat and hen liver.

Two further new metabolites of vinclozolin in the rat were identified. Compound (4) arose from aromatic hydroxylation of (2), and by analogy with the structure of the known metabolite (5), (which was also detected) hydroxylation probably occurred at the 4-position in the aromatic ring. Compound (6) was possibly formed from oxidative cleavage of the vinyl group after hydrolytic opening of the 3,4-bond in the heterocyclic ring. These metabolites were not detected in goat urine. Some evidence was obtained for the presence of the novel aldehyde metabolite (7), in hen liver.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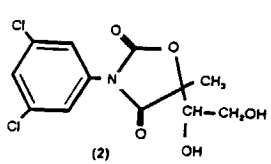
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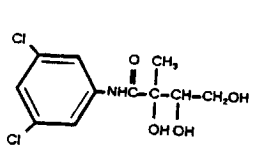
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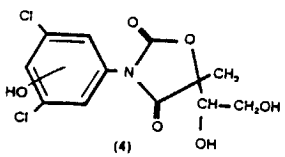
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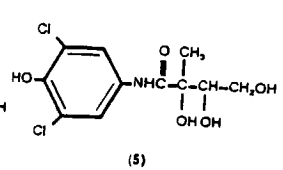
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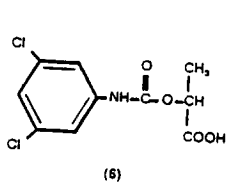
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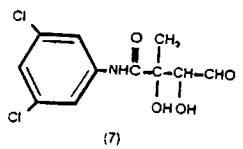
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THE USE OF HUMAN LIVER MICROSOMES IN DRUG DISCOVERY

Maurice Dickins [1], Mary Pudney [2], Vicki S.Latter [3] and Alan T.Hudson [4]

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3-Hydroxy-1,4-naphthoquinone (HNQ) derivatives have been known as potential antimalarial derivatives for a number of years. Their mechanism of action is thought to be due to inhibition of electron transport systems in the malaria parasite. However, a major drawback of these compounds is their susceptibility to metabolic degradation resulting in a loss of in vivo activity. Thus a desirable quality for a compound of this class is resistance to biotransformation in man.

The liver is the principal site of metabolism of many drugs and a number of compounds are metabolised by enzymes present in hepatic endoplasmic reticulum. Liver microsomes were prepared from a number of species including rat, dog, cynomolgus monkey and man. Incubation of the HNQ compound 58C80 (2-(trans-4-t-butylcyclohexyl)-HNQ) with liver microsomes at 37°C in the presence of an NADPH regenerating system resulted in the formation of a more polar metabolite. The metabolite was identified as a hydroxylated derivative of 58C80 and preliminary experiments suggested that the reaction was catalysed by cytochrome(s) P450. The enzymic conversion was partially blocked by classical inhibitors of cytochrome P450-mediated metabolism (CO, SKF-525A and metyrapone) and completely inhibited in the presence of an oxygen depleting system. Marked species differences were observed in the rate and extent of metabolism of 58C80 to the hydroxyl metabolite - man > cynomolgus > dog > rat. It was thought that human liver microsomes may thus be a useful enzyme system for studying the metabolism of this class of compounds with a view to selecting a molecule with a low rate of metabolic conversion.

A series of HNQ compounds, 58C80, 59C80 (2-(trans-4-phenylcyclohexyl)-HNQ) and 566C80 (2-(trans-4-(4-chlorophenyl)cyclohexyl)-HNQ), all of which showed similar antimalarial activity in vitro, were incubated with human hepatic microsomes in the presence of an NADPH regenerating system. Like 58C80, the compound 59C80 was shown to be rapidly and extensively metabolised by human liver microsomes to a more polar derivative. The metabolism was isolated and identified as the p hydroxy derivative of 59C80, suggesting that 59C80 and 58C80 were metabolised by a similar cytochrome P450 isozyme. In contrast, the chlorophenyl compound 566C80 was much less susceptible to degradation by human hepatic microsomes. No metabolites were detectable following incubation of 566C80 with the human liver preparation in the presence of NADPH. These results suggest that both 58C80 and 59C80 would be metabolically degraded in man but 566C80 may be a much more stable derivative and retain activity in vivo.

These results suggest that human liver microsomes may be a useful primary screen for compounds such as HNQs which undergo liver mediated metabolism in man and thus aid drug candidate selection.

GLUCURONIDATION OF ISOMERS OF DIFLUNISAL ACYL GLUCURONIDE IN THE RAT

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Acyl glucuronides have been shown over the past decade to be a class of potentially reactive metabolites, capable of undergoing hydrolysis (regeneration of aglycone), intramolecular rearrangement (isomerisation *via* acyl migration) and intermolecular covalent bonding reactions to protein. We have previously demonstrated the occurrence of such reactions, both *in vitro* and *in vivo*, for diflunisal acyl glucuronide (DAG). Diflunisal (DF), a difluorophenyl derivative of salicylic acid, also forms a phenolic glucuronide and a sulphate conjugate as major metabolites. The present investigation was carried out to determine the biological fate of DAG and its rearrangement isomers (iso-DAG) after their direct i.v. administration to the rat.

DAG (i.e. the biosynthetic 1-*O*-acyl-β-D-glucopyranosiduronic acid) was isolated and purified (preparative HPLC) from the acidified urine of a volunteer who ingested DF. In other urine samples (non-acidified), DAG was permitted to rearrange, and iso-DAG was isolated and prepared as an approximately equimolar mixture of the non-biosynthetic 2-, 3- and 4-*O*-acyl positional isomers. DAG and iso-DAG were each administered i.v. at 10 mg DF equivalents/kg to conscious male Sprague-Dawley rats (5 in each group) previously fitted with catheters in the jugular vein and bile duct.

Systemic hydrolysis (generation of DF and consequent formation of its phenolic glucuronide and sulphate conjugates, as well as partial regeneration of DAG), rearrangement (formation of iso-DAG) and direct excretion were important pathways of DAG disposition in the rat. By contrast, iso-DAG was much more stable to systemic hydrolysis after its i.v. administration at the same molar dose. A group of highly polar metabolites, excreted predominantly in bile, was observed after dosage with either DAG or iso-DAG, and was identified by sequential hydrolyses with β-glucuronidase and alkali as the phenolic glucuronides of iso-DAG (and perhaps of DAG as well). These "diglucuronides" accounted for 36.5 and 12.5% of the doses of iso-DAG and DAG respectively, but were not detected after i.v. administration of diflunisal phenolic glucuronide to a single rat.

The results show that non-biosynthetic iso-DAG is a good substrate for further glucuronidation of the salicylate ring at its phenolic function. The capacity of DAG itself to act as such a substrate is masked by its ready hydrolysis and rearrangement *in vivo*. By contrast, diflunisal phenolic glucuronide appears not to be a good substrate for further glucuronidation of the salicylate ring at its carboxy function.

IN VITRO AND *IN VIVO* ACTION OF MECHANISM-BASED INHIBITORS OF OXIDATIVE DRUG METABOLISM

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The quantitative and qualitative nature of the hepatic microsomal monooxygenase (cytochrome P450) system in an individual liver sample may be characterised *in vitro* using a battery of probe substrates and/or inhibitors. The use of suicide or mechanism-based inhibitors offer a greater potential than most competitive inhibitors due to the irreversible nature of their effect. 1-aminobenzotriazole (ABT) is a suicide inhibitor of P450 acting via production of the highly reactive benzyne which covalently binds to P450 bridging two vicinal N atoms in the protoporphyrin ring system. Chloramphenicol (CAP) inactivates P450 by virtue of the covalent modification of the apoprotein via a lysine residue. We have compared the isozyme specificity of ABT and CAP in hepatic microsomes, from male Sprague-Dawley rats which had either received no pretreatment (C) or had been dosed with an enzyme inducer i.p. once daily for 3 days: phenobarbitone (PB), 80mg/kg and β-naphthoflavone (BNF), 100mg/kg which elevates cytochromes P450IIB and P450I by 40- and 70-fold respectively. In addition we have compared the *in vivo* time course of the mechanism-based inhibition following either ABT (50mg/kg) or CAP (120mg/kg) administration and sacrificing the rats after various times up to 36h.

Oxidative metabolising capacity was assessed by three assays which measured appearance of a fluorimetric metabolite-ethoxycoumarin O-deethylase (ECOD, a non-specific probe), methoxycourmarin O-demethylase (MCOD), a P450IIB specific probe) and ethoxyresorufin (EROD, a P450I specific probe). Immunoblotted microsomes were diluted to give comparable total P450 concentrations to C microsomes for these assays. Inhibitors were studied over a concentration range of 1μM-10mM. A 5 minute preincubation of microsomes with inhibitor and cofactor was carried out prior to substrate addition. In control microsomes both ABT and CAP were potent inhibitors. Concentrations as low as 1μM produced a measurable effect whereas a concentration of 30μM caused 50% inhibition of ECOD. PB and BNF microsomes were less sensitive to ABT requiring a concentration of approximately 100μM for 50% inhibition of ECOD. ABT inhibition of MCOD in C and PB microsomes showed marked similarities (50% inhibition at 70μM), as did inhibition of EROD in control and BNF microsomes (50% inhibition at 80μM). CAP inhibited ECOD and MCOD to similar degrees in C microsomes and these responses were similar to ABT. However there was a marked increase in potency in PB microsomes; the concentration of CAP producing 50% inhibition decreased by an order of magnitude.

In the *in vivo* studies ECOD, MCOD and EROD were maximally inhibited by ABT at 3 hours to 15-20% of control. EROD was fully restored at 12 hours, ECOD at 36 hours whilst MCOD was still depressed at 36 hours. CAP inhibition was also maximal at 3 hours although the effect was substantially less than ABT (30-50% of control). Recovery from CAP was uniformly fast and complete by 24 hours. Hence both ABT and CAP are effective mechanism-based inhibitors both *in vitro* and *in vivo* showing different patterns of selectivity towards P450 isozymes.

INFLUENCE OF THE SHO-SAIKO-TO (TJ 9) ON THE PENTOBARBITAL SLEEPING TIME AND ON THE KINETICS OF ANTIPYRINE AND THEOPHYLLINE - INTERACTION WITH THALLIUM

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One of the Japanese Kampo medicines Sho-saiko-to (TJ 9) comprises 7 crude drugs. The actions of this preparation include immunomodulation, protection against liver cell injury caused by various agents, antiinflammatory and metabolic effects etc. In the experiments performed in male mice (25-28g b.w.) the influence of single or repeated administration of TJ 9 on the pentobarbital sleeping time (PST) and antipyrine or theophylline serum level (ASL or TSL) was investigated. ASL and TSL was determined after the extraction step by HPLC. Pretreatment with a single dose of TJ 9 12h before the administration of drugs did not change significantly the parameters examined. However, TJ 9 given p.o. once daily for 4 days (250 or 500 mg/kg) shortened the PST. The body weight and the weight of liver were followed in this experiment. Hepatotoxic agent thallium acetate (Tl) applied 48h before the experiments prolongs the PST and elevates the ASL and TSL. The multiple administration of TJ 9 (400 mg/kg p.o. once daily over 4 day period) before the Tl p.o. administration decreased especially the toxic effect of Tl on the PST. Similarly the thallium induced lipoperoxidation and the depletion of glutathione (GSH) in the liver homogenates was also prevented by TJ 9 administration. Lipoperoxidation expressed as amount of malondialdehyde was measured in liver homogenates of treated mice by thiobarbituric acid test. The glutathione level (GSH) was estimated in the same tissue homogenates. The favourable effect of TJ 9 is dose depending. The high doses are less effective. The repeated p.o. administration of TJ 9 prevented also the acute Tl intoxication.

The authors wish to thank Tsumura a. Comp., Tokyo 102, Japan, for the kind supply with TJ 9.

STUDIES ON THE METABOLISM OF H 234/09-[^{3}H] – A NEW CLASS III ANTIARRHYTHMIC DRUG – IN RAT

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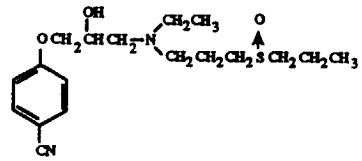
H 234/09 has been selected in a project pursuing the development of compounds with class III electrophysiological activity. The compound prolongs the action potential duration and therefore the refractory period in cardiac tissue in various animal models and man.



The aim of the present investigation was to study the excretion pathways, the pattern of urinary metabolites and to identify the main metabolites in urine after a single iv and oral dose of H 234/09-[^{3}H](50 μmol/kg) to male and female rats (n=8).

After 96 h the averaged total recovery of the dose was 90% and 76-94% was excreted within the first 24 hours. About 55% of the dose was found in urine after both an iv and oral dose. Based on liquid chromatographic retention times, there was no difference in the urinary pattern in male and female rats and the profiles were similar after both routes of administration. Unchanged H 234/09 and its metabolites were isolated from urine by extraction with dichloromethane/ethylacetate (1:3) at pH 10. About 70% of excreted radioactivity was recovered by solvent extraction and this fraction was further purified by an Advance Automated Sample Processor (AASP®) with C-18 extraction columns. The AASP® was combined with an isocratic HPLC system allowing for automatic collection of LC fractions after repeated injections. The metabolites were identified by thermospray LC-mass spectrometry including daughter ion scan, ^{1}H-NMR and synthetic references.

Spectral evidence for metabolite identification will be presented and biotransformations encountered so far comprise sulfoxide oxidation, aliphatic hydroxylation and oxidative N-dealkylation.

THERMODYNAMIC ASPECTS OF SUBSTRATE ENANTIOSELECTIVITY IN THE ESTERASE-CATALYSED HYDROLYSIS OF 2-PHENYLPROPIONIC ACID ESTERS

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The (R)- and (S)-enantiomers of methyl 2-phenylpropionate and phenyl 2-phenylpropionate were used as model substrates of pig liver carboxylesterase (EC 3.1.1.1); the steady-state kinetics of the reaction was investigated over a range of temperatures between 293 K and 308 K. The data obtained were interpreted in terms of the simplified equation

$$E + S \overset{K_m}{\rightleftharpoons} ES \overset{k_{cat}}{\rightarrow} E + P_1 + P_2$$

where K_m represents the Michaelis-Menten constant and k_{cat} may be identified as the first order rate constant of formation of the acyl-enzyme intermediate.

For both esters, plots of $\ln(k_{cat})$ against $1/T$ were linear, and higher k_{cat} values were found for the (R)-enantiomers. No linear behaviour of K_m as a function of temperature was observed. A moderate but significant enantioselectivity, as assessed by the enantiomeric ratio (k^R_{cat}/k^S_{cat}) was established for both esters. At lower temperatures, enantioselectivity of the phenyl ester was about two to three times larger than that of the methyl ester.

The thermodynamic formulation of the Transition State Theory (1) enabled the calculation of the enthalpy ($\Delta H^{\#}$) and entropy ($\Delta S^{\#}$) changes determining (at a given temperature) the value of k_{cat}. Thus, for the phenyl ester the following observations were made.

$\Delta H^{\#}$ for the (R)-enantiomer being about 50% of that for the (S)-enantiomer ($\Delta\Delta H^{\#} \equiv 45$ kJ/mol), the relative enthalpy effects clearly favour preferential hydrolysis of the (R)-enantiomer. However, as $\Delta S^{\#}$ for the (R)-enantiomer (the "better" substrate) is larger than $\Delta S^{\#}$ for the (S)-enantiomer, the entropy changes (partially) compensate for enthalpy effects, resulting in a difference in height of the energy barriers of about 4 kJ/mol.

For the methyl ester, the $\Delta\Delta H^{\#}$ and $\Delta\Delta S^{\#}$ values were smaller than those for the phenyl ester, but the same trend was observed.

Literature

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IS THE CYNOMOLGUS HEPATIC MICROSOMAL SYSTEM A POTENTIAL IN VITRO MODEL FOR METABOLIC GENETIC POLYMORPHISM IN MAN. STUDY WITH METOPROLOL.

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Due to the problems encountered in obtaining human liver samples it would be useful to have a working animal model capable of predicting genetic polymorphism in human very early on in the development of a new drug. Competitive inhibition studies with microsomes from the macaca fascicularis monkey have shown that kinetic constants of dextrometorphan O-demethylation and debrisoquine- 4-hydroxylation are similar to those obtained with human microsomes. Metoprolol (M), like many other β-blockers, is administered to human as a racemate and O-demethylation and α-hydroxylation are stereoselective in man.
The aims of this study were the following : does the cynomolgus primate metabolize stereoselectively metoprolol and does quinidine stereoselectively inhibits O-demethylation and α-hydroxylation ?
Metoprolol, α-hydroxymetoprolol and O-demethylmetoprolol enantiomers were separated by reversed-phase HPLC after derivatization with naphthylethyl-isocyanate in order to enhance sensitivity of detection .
Metoprolol enantiomers were metabolized by monkey hepatic microsomes with similar Michaelis constant (Km = 30 and 37 µM and Vm = 3,4 and 4,5 $nmole.mg^{-1}.min^{-1}$ for R(+) and S(-), respectively). As expected the preferential metabolism of S(-)M was coupled with the greater formation of the S(-) O-demethyl metabolite.
Quinidine inhibited a). partially the O-demethylation : even at 100 µM not more than 80 % of the O-demethylation could be inhibited (metoprolol = 10 µM) b). the stereoselective metabolism of metoprolol.The metoprolol R/S ratio at the end of a 30 min incubation was 1.72 without quinidine, 1.24 and 1.09 with quinidine, 1 and 100 µM, respectively.
In a competitive inhibition study between metoprolol and dextrometorphan, M (50 µM) was able to inhibit 50 % of the dextrometorphan metabolism.
These results indicate that cyt. P450 IID is involved in the metabolism of metoprolol by cynomolgus microsomes, and suggest that this animal species may be of some predictive value for human debrisoquine polymorphism. However, as its happens for many models more work is needed to better define its limits of application.

METABOLISM AND PHARMACOKINETICS OF DINALINE AND ITS METABOLITE ACETYLDINALINE IN MICE, RATS, MONKEYS, AND DOGS

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Dinaline (4-amino-N-(2'-aminophenyl)benzamide) and acetyldinaline (4-acetylamino-N-(2'-aminophenyl)benzamide) are potential anticancer agents with activity against tumors normally refractory to currently-known agents. Previous studies showed that dinaline is transformed to acetyldinaline.

A major aim of the present studies was to investigate the behavior of the metabolite in comparison with the parent compound and to determine whether acetylation is a reversible process.

Dinaline was administered to mice (IG: 467.5 mg/kg, LD_{50}), rats (IG: 2, 10, and 50 mg/kg, IV: 10 mg/kg), monkeys (IG: 5 mg/kg), and dogs (IG: 5 mg/kg, IV: 5 mg/kg). Acetyldinaline was administered to rats (IG: 2, 10, 50 mg/kg, IV: 10 mg/kg) and to monkeys (IG: 5 mg/kg).

Plasma was collected and analyzed for dinaline and its metabolite acetyldinaline by solid phase extraction and subsequent HPLC analysis using a selective and sensitive plasma HPLC assay.

Dinaline was rapidly absorbed in all species examined and extensively metabolized to acetyldinaline except in the dog. In dogs no N-acetylation products were found after IV and IG administration of 5 mg/kg doses of dinaline.

Following dinaline administration to rats, a fast and saturable conversion to acetyldinaline was found leading to nonlinear kinetics of dinaline in plasma.

In monkeys, only low concentrations of dinaline were found up to 3 h postdose following dinaline administration.

In dogs, no acetyldinaline was found. This demonstrated the lack of N-acetylation in this species.

The pharmacokinetics of acetyldinaline was dose-linear following acetyldinaline administration to rats.
No dinaline was found in rats and monkeys following administration of acetyldinaline.
This behavior suggests that, in this case, N-acetylation is not a reversible process or equilibrium is on the side of the acetylation product.

IN VITRO METABOLISM OF DINALINE AND ACETYLDINALINE BY HUMAN LIVER

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Dinaline (4-amino-N-(2'-aminophenyl)benzamide) and acetyldinaline (4-acetylamino-N-(2'-aminophenyl)benzamide), its major in vivo metabolite in several species, are investigational anticancer drugs with activity against some tumors normally refractory to currently known agents. The aim of the present investigation was to study the in vitro biotransformation of dinaline and acetyldinaline by human liver subcellular fractions in relation to the genetic polymorphism of arylamine N-acetyltransferase (NAT) enzyme activity in man.

The acetyl CoA-dependent N-acetylation of dinaline was measured in liver cytosols from six human livers which had been previously characterized according to their rates of metabolism of the polymorphic NAT substrate sulfamethazine (SMZ). At the same time, microsomal deacetylation of acetyldinaline was compared to that of the carcinogen 2-acetylaminofluorene (AAF). All enzyme measurements were obtained using a specific and sensitive HPLC assay to measure product formation rates. Dinaline-NAT activity was generally high among the six human liver cytosols, but showed no relationship to the N-acetylation of SMZ, suggesting that "acetylator phenotyping" with currently-established methods would not predict the in vivo extent of dinaline N-acetylation. Kinetic characterization of dinaline-NAT activity using both partially purified human liver NAT isozymes and recombinant expressed products of two cloned human NAT genes, NAT1 and NAT2 (Blum et al., DNA 9:193-203, 1990), demonstrated that dinaline is N-acetylated more efficiently by the "monomorphic" NAT1 enzyme than by the "polymorphic" NAT2. Acetyldinaline was not detectably deacetylated by human liver microsomes possessing high AAF deacetylase activity, consistent with in vivo pharmacokinetic studies of its disposition.

BEAUVERIA BASSIANA. CHARACTERISATION OF A FUNGAL MODEL OF MAMMALIAN DRUG METABOLI

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The concept of using microorganisms to model mammalian drug metaboli
proposed by Smith and Rosazza (1974) is now well established (Davi
1988). The fungal organism Beauveria bassiana is able to extensivel
transform several drugs to produce metabolites similar to those produce
by mammalian systems (Griffiths et al 1990). The present research
programme aims to characterise the drug metabolising enzyme system of
this organism to facilitate its use as a model system.

Three substrates; diazepam, warfarin and testosterone were chosen for
these studies. Previous work in this laboratory had identified simple
growth and biotransformation conditions for metabolism of the substrates
by Beauveria bassiana. Growth in a simple defined medium followed by
biotransformations by resting cells in buffer were found to be most
successful and convenient. The results presented here describe the
kinetics and inhibition of biotransformations by whole cell suspensions
and provide evidence for the nature of the enzyme(s) involved.

Biotransformations of the three substrates were inhibited by the
classical cytochrome P-450 inhibitors SKF-525A and metyrapone, thereby
providing evidence for the involvement of this enzyme system in these
conversions. The extent of biotransformation in the presence of these
inhibitors increased with increasing lipophilicity of the substrate. In
addition, apparent Km values decreased with increasing lipophilicity of
the substrate. Substrate inhibition was observed in diazepam metabolism
above 0.1mM, in contrast to the metabolism of the other two substrates
which exhibited linear kinetics to concentrations as high as 5.0mM.
Inhibition experiments, lipophilicity effects and diazepam substrate
inhibition all parallel xenobiotic biotransformations by mammalian
hepatic cytochrome P-450.

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STEREOSELECTIVE METABOLISM OF INDOPROFEN

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Indoprofen (2-[p-(1-oxo-2-isoindolinyl) phenyl] propionic acid); IP is a 'profen' previously used as a general antiinflammatory and analgesic, mainly in the treatment of rheumatic and related disorders. IP has a chiral carbon atom and can thus exists as a pair of eriantiomers and its pharmacological activity is almost entirely due to (+)-S-IP. Unlike other profens, it seems not to undergo metabolic chiral inversion.

We describe here studies on the stereoselectivity of metabolism of IP in rats and mice. Intact and bile duct-cannulated rats were orally dosed with ^{14}C-*rac*-IP (20 mg/Kg, 5 mCi/rat) and mice were orally dosed with ^{14}C-*rac*-IP (25 mg/Kg, 5 mCi/mouse). Urinary, faecal and biliary metabolites were separated by HPLC and quantitated by liquid scintillation counting. The enantiomeric composition of IP after dosing the racemate was determined after derivatization with L-leucinamide.

There was a complete recovery of *rac*-IP in 0-72h (rats) and 0–96h (mice) (29% in urine, 71% in faeces for rats and 73% in urine,31% in faeces for mice). *Rac*-IP was recovered in rats largely as free IP over 0-48h (7.6% in urine, 25.3% in faeces) and also as free 5- and 6-hydroxylated IP (6.0% in urine, 17.9% in faeces). There was little conjugation. Rat bile contained free IP and probable rearranged isomers of IP glucuronide. *Rac*-IP was recovered in mice as free IP (13.2% in 0-48h urine and 11.6% in 0–24h faeces) and its glucuronide (24.6% in 0-48h urine and 0% in faeces) and also as free 5 OH-IP (18.4% in 0-48h urine and 3.7% in 0-24h faeces). Enantiomeric analysis of rat urine, faeces and bile of rats given *rac*-IP after hydrolysis of conjugates showed the S–isomers of IP and of OH-IP to be excreted predominantly in the urine but essentially identical excretion of both enantiomers in faeces and bile.

ISOLATION OF 4-HYDROXYANDROSTENEDIONE-4-O-GLUCURONIDE FROM RABBIT URINE AND PREPARATIVE SYNTHESIS IN VITRO

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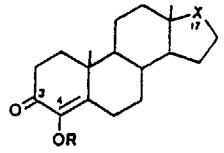
4-Hydroxyandrostenedione (4-HAD, I) is a potent aromatase inhibitor and currently under clinical investigation for treatment of metastatic breast cancer. After per-oral administration I is well absorbed and extensively metabolized in animal and man. The 4-O-glucuronide of 4-HAD (Ia) has been identified as a major metabolite and was required as a standard for clinical pharamacokinetic investigations. The glucuronide Ia was isolated from the urine of 4-HAD-treated rabbits and from incubations of 4-HAD with rabbit liver subcellular fractions.

Two male rabbits received 600 mg/kg 4-HAD perorally. Analysis of the 0-24 h urine by HPLC and TLC combined with specific enzymatic cleavage (ß-glucuronidase) revealed the presence of three glucuronides (Ia, IIa and IIIa) which were isolated by preparative reversed phase HPLC. IIa and IIIa were identified as the 4-O-glucuronides of 4-hydroxy-17-α-testosterone (II) and 4-hydroxy-17-ß-testosterone (III) by FAB mass, ^{1}H-NMR- and UV-spectroscopy.



I R = H Ia R = gluc X = 
II R = H IIa R = gluc X = 
III R = H IIIa R = gluc X = 

Alternatively 60.5 mg of 4-HAD were incubated in the presence of 48 mM UDPGA with a 4.5xg supernatant from a rabbit liver homogenate at 37 °C and pH 8.0. After 10 h of incubation 4-HAD was completely glucuronidated without significant side product formation. 63 mg (yield: 63%) of Ia were isolated by HPLC and its identity with the 4-O-glucuronide from urine was verified.

The results show that 17-keto reduction was a significant metabolic pathway in the rabbit. The excretion of the glucuronides of the reduction products complicated isolation of Ia. This was not the case with in vitro incubations, these resulted in an almost quantitative formation of Ia, which could be isolated with a high yield. This exemplifies the usefulness of in vitro systems for production of glucuronide conjugates.

BIOTRANSFORMATION OF THE AROMATASE INHIBITOR CGS 16 949 A IN RAT AND DOG IN VIVO AND IN FIVE SPECIES IN VITRO INCLUDING MAN.

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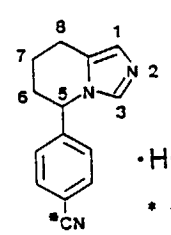
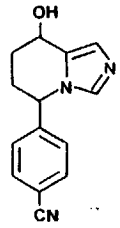
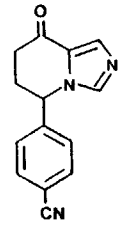
CGS 16 949 A (I) is a potent, non-steroidal selective inhibitor of the aromatase enzyme system in vitro and of estrogen biosynthesis in vivo. The compound is currently undergoing clinical trials for the treatment of metastatic breast cancer.



CGS 16 949 A (I)



II



III

The biotransformation of [^{14}C]-CGS 16 949 A was studied in vivo in rats and dogs, and in vitro using freshly isolated hepatocytes from dogs and marmosets and liver microsomes of rat, dog, marmoset, baboon and human. [^{14}C]-I was rapidly and extensively metabolized in rats (5 mg/kg) and dogs (1 mg/kg) after i.v. and p.o. administration. The main metabolic pathway was stereoselective oxidation to the 8-trans-hydroxy derivative II followed by O-glucuronidation. Rat urine contained II (36% of dose), the 8-keto metabolite III (4%), the 8-O-ß-D-glucuronide of II (7%), and unchanged preparation (7%). Dog urine mainly contained the 8-O-ß-D-glucuronide of II (47%).

In liver microsomes of all species studied, I was oxidized with high stereoselectivity to the 8-trans-hydroxy metabolite II. The cis-diastereomer of II and the 8-keto metabolite III were formed in low amounts together with two additional minor unidentified metabolites. In marmoset hepatocytes as well as in liver homogenate in the presence of UDPGA, the quaternary 2-N-glucuronide of I was produced. This metabolite was not formed with liver homogenates of the other species. Therefore the marmoset may be not a suitable model for the metabolism of I in man.

GLUCURONIDATION OF 3'-AZIDO-3'-DEOXYTHYMIDINE (AZT)

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Glucuronidation of 3'-azido-3'-deoxythymidine (AZT) was characterized *in vitro* in man and in various animal species. The glucuronide isolated from liver microsomes, was identified by fast atom bombardment and desorption in chemical ionization mass spectrometry. AZT glucuronidation in liver microsomes of man and monkey proceeded similarly with an apparent V_{max} of 0.98 nmol/min/mg protein and apparent K_m of 13 mM. Drug interference experiment revealed that probenecid decreased hepatic AZT glucuronidation *in vitro* (I_{50} = 1.5 mM), whereas paracetamol did not exert any effect at concentrations up to 21.5 mM. Inter-species comparison indicated that AZT was glucuronidated at a highest rate in man and in monkey (0.50 nmol/min/mg protein); pig and rat glucuronidated the drug 2 and 3 times less, respectively. When compared to the congenic normal strain, Gunn rats, which are deficient in bilirubin UDP-glucuronosyltransferase, could form AZT glucuronide at a similar extend (0.18 nmol/min/mg protein). In rats, AZT glucuronidation was stimulated 4-fold by phenobarbital, whereas 3-methylcholanthrene or clofibrate failed to increase this activity. This result was consistent with the bulkiness of AZT molecule (thickness 6.7 Å), which is a critical structural factor for glucuronidation of the drug by phenobarbital-induced isozymes. All together, the results strongly suggest that UDP-glucuronosyltransferase (phenobarbital inducible forms) is responsible for AZT glucuronidation (1).

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Acknowledgements:

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PLASMA AND TUMOR TISSUE CONCENTRATIONS OF DINALINE, ACETYLDINALINE, AND METHYLDINALINE IN RATS FOLLOWING EQUIMOLAR ORAL DOSES OF THE THREE COMPOUNDS

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Dinaline (4-amino-N-(2'-aminophenyl)benzamide) and chemical derivatives acetyldinaline (4-acetylamino-N-(2'-aminophenyl)benzamide) and methyldinaline (4-methylamino-N-(2'-aminophenyl)benzamide) are potential anticancer agents displaying activity against some tumors normally refractory to current drug therapy. Acetyldinaline is the major metabolite of dinaline and methyldinaline.

The aim of the present study was to investigate the penetration of dinaline, acetyldinaline, and methyldinaline into tumor tissue.

Solutions of equimolar doses (12.3 µmol) of ^{14}C-dinaline, ^{14}C-acetyldinaline, and methyldinaline were administered by oral gavage to tumor-bearing rats. Plasma and tumor tissue were collected and analyzed for the three compounds by solid phase extraction and subsequent HPLC analysis using a selective HPLC assay.

Following administration of dinaline, all dinaline plasma concentrations except that of the first time point (0.25 h) were below the detection limit of 0.1 µg/mL. Plasma and tumor tissue Cmax values for acetyldinaline were 11.4 µg/mL and 8.28 µg/g, respectively.

Following acetyldinaline administration, no plasma concentrations of dinaline above the quantification limit could be detected. Plasma and tumor tissue Cmax values for acetyldinaline were 13.7 µg/mL and 6.36 µg/g, respectively.

Following methyldinaline administration, the plasma and tumor tissue Cmax values for methyldinaline were 3.81 µg/mL (0.25 h) and below 0.5 µg/g, respectively. No detectable plasma concentrations of methyldinaline were present 8 h postdose. No plasma and tumor concentrations of dinaline above the quantification limit could be detected at any time point. The acetyldinaline plasma and tumor Cmax values were 4.43 µg/mL and 2.46 µg/g, respectively. Thus, methyldinaline appears to be rapidly metabolized with the highest unchanged plasma concentrations occurring at 0.25 h postdose. These results suggest that the rate of the N-demethylation is slower than the N-acetylation step.

Acetyldinaline was the major compound detected in plasma and tumor tissue following equivalent single IG doses of dinaline, acetyldinaline, and methyldinaline. Tumor acetyldinaline concentrations of approximately one-half the plasma concentrations are obtained following equivalent single IG doses of dinaline, acetyldinaline, and methyldinaline. The constant ratio between plasma and tissue acetyldinaline concentrations suggests a fast penetration rate of acetyldinaline into tumor tissue.

COMPARATIVE METABOLISM OF ^{14}C-DINALINE AND ^{14}C-ACETYLDINALINE IN MICE, RATS, AND MONKEYS

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Dinaline (4-amino-N-(2'-aminophenyl)benzamide) is an investigational anticancer drug with activity against some tumors normally refractory to currently-known agents.
Acetyldinaline (4-acetylamino-N-(2'-aminophenyl)benzamide), which possesses a similar therapeutic spectrum, is the dominant metabolite in mice, rats, and monkeys.
To obtain metabolic profiles and to determine route of elimination, oral doses of dinaline and acetyldinaline were administered to mice, rats, and monkeys.

Mice, rats and monkeys were housed in metabolic cages and fasted overnight prior to dosing. ^{14}C-dinaline and ^{14}C-acetyldinaline were administered intragastrically by gavage (mice: 10 mg/kg, rats: 10 mg/kg, monkeys: 5 mg/kg). Radioactivity was measured using liquid scintillation counting and metabolic profiles were obtained using a gradient HPLC method with on-line radioactivity detection.

Over a 168-h collection period following a single oral dose of dinaline and acetyldinaline to rats, 64.0% and 62.1% of the radioactive dose were excreted in urine and 27.5% and 30.1% in feces, respectively. The major component in the 0-8 h fraction in both cases was acetyldinaline, comprising 62.9% and 65.9% of the radioactivity excreted in urine. The residual radioactivity in liver and kidney 168 h postdose represented 0.022% and 0.0025% of dose for dinaline and 0.019% and 0.0021% for acetyldinaline, respectively.
In monkeys, the radioactivity excreted renally over a 48-h period following single oral doses of dinaline and acetyldinaline was 75.8% and 56.0%, respectively. The major component in the 0-8 h fraction in both cases was also acetyldinaline, comprising 78.7% and 80.2% of the radioactivity excreted in urine.
In mice, the radioactivity excreted renally over a 48 h period following single oral doses of dinaline and acetyldinaline was approximately 42% and 63%, respectively. The major component in the 0-8 h fraction in both cases was also acetyldinaline, comprising 38.7% and 69.1% of the radioactivity excreted in urine.

After intragastric administration of radiolabelled dinaline and acetyldinaline to mice, rats, and monkeys, radioactivity was rapidly excreted, predominantly via the renal route. Metabolic profiles were similar both intraspecies and interspecies for the two compounds following oral administration. Acetylation seems to be slower in mice than in rats or monkeys. In all cases, acetyldinaline is the main compound excreted.

METABOLITE PATTERN OF MOCLOBEMIDE IN RATS: COMPARISON OF IN-VIVO AND IN-VITRO RESULTS.

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Moclobemide (p-chloro-N-(2-morpholinoethyl)benzamide) belongs to a new generation of moroamine oxidase inhibitors and is a promising drug for the treatment of depression [1-3]. The aim of the study presented here was to investigate the metabolite patterns of moclobemide in bile duct-cannulated rats and to compare the results with those obtained in a preparation of the isolated perfused rat liver (IPL). Moclobemide, labelled with ^{14}C in the carboxamide group, was administered in-vivo intraduodenally (50 mg/kg) and in-vitro via perfusate (15.3 mg). In rats bile and urine and in the IPL bile and perfusate were collected for 48 and 2 hours, respectively. The metabolite patterns were investigated by two-dimensional TLC autoradiography before and after enzymatic hydrolysis. The disposition of total radioactivity as well as the metabolite patterns in bile, urine and perfusate revealed distinct parallels between in-vivo and in-vitro results. The similarities of the metabolite patterns observed in bile, when comparing in-vivo and in-vitro results, were greater than those of urine and perfusate, as may be anticipated. Specific differences of the metabolite patterns seen in-vivo, when comparing urine and bile, were retained in-vitro, when comparing perfusate and bile. In conclusion a closely controlled model of the IPL was established. Using moclobemide as a test substance the model was shown to be well suited for the investigation of the disposition and biotransformation of foreign compounds.

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3-ARYLHYDANTOINS - BIOTRANSFORMATION OF THE HYDANTOIN RING

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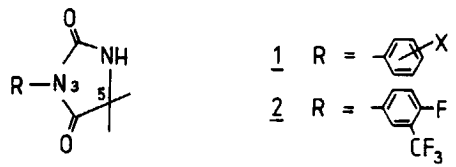
Within the 3-arylhydantoins 1 the 5.5-dimethyl-3-(α,α,α,4-tetrafluor-m-tolyl)-hydantoin (2) exhibited an interesting



1 R =

2 R =

antischistosomal activity against the strains Schistosomia mansoni, S. haematobium and S. japonicum [1].

After single oral and intraperitoneal administration of the compound 2, labelled with ^{14}C in the C-5 position of the hydantoin ring, to mice, the recovery of total radioactivity in urine and feces with 60-70 % and in the organs and the carcass with 1-2 % of the administered dose was obviously incomplete. Subsequently the detection and determination of $^{14}CO_2$ in the respiratory air, accounting for 20-25 % of the administered dose was surprising, since the C-5 position of the hydantoin ring, which carries the ^{14}C-label, is shielded by the two substituents, the methyl groups. Nevertheless, the elimination of the label as $^{14}CO_2$ alloved the conclusion that the hydantoin moiety undergoes an intensive oxidative biodegredation with ring opening and decarboxylation.

The isolation and identification of the major metabolites in mice urine led to the following postulate of the biotransformation pathway : Beginning with the hydroxylation of the methyl groups and further oxidative degredation a ring-opened C-5 carboxylic acid must be formed, which is further decarboxylated, resulting in the liberation of C-5 as $^{14}CO_2$.

Thus it could be demonstrated, that C-5 of the hydantoin moiety plays an important role in the intensive metabolisation of the hydantoin ring.

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RAMIPRIL: AUTORADIOGRAPHIC STUDIES ON TRANSFER AND BINDING SITES OF (^3H) RAMIPRILAT IN THE RAT BRAIN

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RAMIPRIL (HOE 498) is a new angiotensin converting enzyme (ACE) inhibitor. The compound is an esterified prodrug which, upon absorption, is hydrolysed to the pharmacological active metabolite, the dicarboxylic acid RAMIPRILAT.

While the role of the peripheral renin angiotensin system (RAS) with respect to blood pressure control is well documented, many questions remain still open concerning the brain RAS. Since the central system is separated from the peripheral one by the blood brain barrier, the ability of a converting enzyme inhibitor to cross this natural barrier to reach the brain is an essential prerequisit for efficacy.

The brain transfer studies were performed in healthy female rats which were dosed three times intravenously with 56 MBq Ramiprilat-^3H/kg b.w.. The quantitative evaluation of the autoradiograms was carried out by videodensitometry using an automatic picture analysis device.

The autoradiograms revealed radioactivity in the brain shortly after the last dose. Amongst others it was detected in defined brain areas known from in vitro receptor binding studies for their ACE content, e.g. corpus striatum, substantia nigra, thalamus, and in the choroid plexus.

From the fact that intravenous administered RAMIPRILAT is able to penetrate the blood brain barrier it can be concluded that this is also the case for RAMIPRILAT which is formed immediately after intestinal absorption from orally given RAMIPRIL.

LYSOSOMOTROPIC DRUGS TARGETTING IN LIVER CELLS

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Lysosomotropic drugs are taken up selectively into lysosomes during in vivo administration, some of them cumulate by liver macrophages (suramin). Lysosomotropic agents suppressing the rate of intralysosomal proteolysis can be considered as a model resembles some symptoms of inherited disorders. Chloroquine is known lysosomotropic and acidotropic drug, which alters the recycling of receptors for lysosomal enzymes, transferrin; this drug is used for suppression of intralysosomal proteolysis in isolated cells (hepatocytes, macrophages). It was shown that in vitro chloroquine inhibited purified enzymes - cathepsins B, L, H reversibly in concentrations less than observed inside rat liver lysosomes in vivo. On the contrary administration of high doses of chloroquine induced increase of both cysteine and aspartic proteinase activity. The prominent effect was noted in case of repeated drug administration. In case of single chloroquine administration (50-70 mg/kg) the peak of drug accumulation in liver was noted 0.5 - 1 h after with the almost total elimination up to 24 h. During repeated chloroquine injections in the same dose (7 times) similar concentrations of drug were observed in liver cells, but elimination of the drug was much slower (up to 72 h). Intralysosomal chloroquine accumulation was followed by lysosome swelling; the drug was bound mainly with membrane fraction. The increase of aspartic and cysteine (less) proteinases activity possibly was related to intralysosomal storage and secondary changes of the structures.

It's known that in vivo chloroquine was cumulated by liver and muscle tissues. Some complications during pharmacotherapy by this drug can be result of lysosomotropic action of chloroquine, especially during chronic treatment.

IDENTIFICATION OF CONJUGATED METABOLITES OF MORPHINANIUM ENANTIOMERS AFTER RAT LIVER PERFUSION WITH IONSPRAY LC/API-MS

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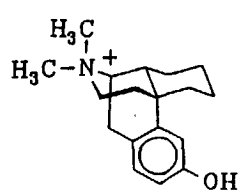
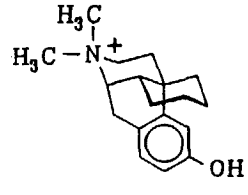
On studying stereoselective pharmacokinetics and metabolism in a rat liver perfusion with ^{14}C-labelled quaternary ammonium compounds N-methyldextrorphan and N-methyllevorphanol, we observed the formation of three metabolites.



N-methyllevorphanol



N-methyldextrorphan

One of these could be hydrolyzed by β-glucuronidase. The others could not be hydrolyzed by β-glucuronidase nor by arylsulphatase. To further analyze these metabolites we used ionspray LC/API-MS(1), a method which has proven to be successful for these quaternary ammonium compounds: No thermal degradation of the quaternary ammonium group occurred. To distinguish metabolites and parent compound from endogenous compounds, we made use of compounds labelled with the stable isotope deuterium. We carried out a rat liver perfusion with a 1:1 mixture of ^{2}H-labelled N-methyldextrorphan and unlabelled N-methyldextrorphan and analyzed the samples of bile and perfusate with LC-MS. HPLC was done on a Spherisorb S5C6 column (150 mm * 2 mm I.D.). Gradient elution was employed with an ammonium acetate buffer and acetonitrile. After UV detection the eluate was led through a splitter, which allowed ca. 1% of the eluate to flow into the ionspray LC/MS interface and to the atmospheric pressure ion source of a NERMAG R 3010 triple quadrupole mass spectrometer. With this method m/z-values of the intact metabolites could be determined and two metabolites could be identified as a glucuronide conjugate and a glutathione conjugate, respectively. Also, data on N-methyllevorphanol will be presented.

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METABOLISM OF SEVERAL ERGOTS IN RAT CULTURED HEPATOCYTES. COMPARISON OF CQA BIOTRANSFORMATION IN YOUNG AND OLD RATS.

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The biotransformation of CQA 206-291 has been studied in rat primary hepatocyte cultures to elucidate the in vivo first pass effect and bioavailability. Additionally the metabolism of CB 154, SDZ 208-912, CU 32-085 and CQP 201-403 have been investigated.

Hepatocytes were isolated from male Wistar rats after liver perfusion with collagenase. Cells were seeded on nu-serum coated plates at a final density of 5×10^5 viable cells/ml and maintained in culture with Complete Medium (DEMEM:F12 plus 0.2% bovine albumine, 2% nu-serum, antibiotics, hormones and ligands). Medium was changed 45-60 minutes after seeding and was replaced by fresh medium containing the drugs (10 µM). Hepatocyte cultures were exposed to the drugs from 0 to 48 hours.

Rat cultured hepatocytes metabolized CQA extensively with respect to turnover (90 and 98% of total metabolites formed after 24 and 48 hours incubation respectively) as well as regarding the number of metabolites formed. The quantitation of the metabolites and the parent drug was based on the integration of radioactive peaks obtained after HPLC separation of the metabolites. Based on retention time and/or metabolites available as reference compounds several peaks can be assigned tentatively to metabolites produced in vivo in rat. In general rat cultured hepatocytes exibited a high turnover for each ergot investigated with the exception of 208-912. The percentage of parent drug in the medium after 24 hours incubation for CQA, CB 154, CU and CQP (10, 16, 5 and 11 respectively) correlated well with data from animal studies in rat, where a low bioavailability (6%, 1%, <5% and 5%) and high first pass effect was observed. The ergot 208-912 is known to have a low first pass effect and high bioavailability in vivo in the rat (58%). The percentage of the parent drug after 24 hours of incubation in rat cultured hepatocytes was 55%. These data show that drug turnover in rat cultured hepatocytes is a predictive measure for in vivo first pass and drug bioavailability and consequently a useful way to evaluate these parameters for newly developed compounds.

On the basis that a decline in hepatic drug metabolism has been shown with senescence in rats, the metabolism of CQA was compared in cultured hepatocytes obtained from young and old rats. We have demonstrated that CQA turnover after 24 hours of incubation is lower in aged than in young rats, and that the half-life of CQA in the culture medium is increased in the aged rat (17.8 h) compared with the value obtained in young rats (7.5 h). The kinetics of CQA metabolism may be altered in the aged rats and is currently being investigated.

THE METABOLISM OF CHINOIN-175 IN THE RAT

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CHINOIN-175 is a selective MAO-B inhibitor which also inhibits the uptake of dopamine. Its inhibiting potency appeared to be similar to that of deprenyl both in vitro and in vivo.
Previous studies with CHINOIN-175 have revealed that MAO-B blocking effect can be primarily attributed to the unchanged drug, but the metabolites formed could also be biologically active compounds. We therefore studied the metabolism of the ^{14}C labelled CHINOIN-175 in the plasma and urine of rats after single oral doses.
The oral dose of CHINOIN-175 is well absorbed from the gastrointestinal tract of rats, the bioavailability of the compound is 100 %. Seventy-five to 80 % of the radioactive dose is eliminated in the urine.
For the determination of the metabolic pattern of 0-24 hour urine the compounds were separated by TLC. It revealed extensive metabolism of the original compound. Five percent of the radioactivity eliminated via the urine was the unchanged molecule.
The structure of plasma and urinary metabolites were identified by MID mass spectrometry after derivatisation and capyllary GC separation and comparison with reference materials. The major metabolites were found to be N-dealkylated compounds (p-F-amphetamine - 19 D%, p-F-methamphetamine - 16 D%, p-F-desmethyl-deprenyl - 1 D%). The identified structures are also biologically active molecules, but it should be stressed, that the original compound is an L (-) optical isomer and as during the formation of the metabolites no change take place on the chiral carbon atom, the metabolites are also L (-) optical isomers, which have one order of magnitude weaker effect on the central nervous system than the D (+) isomers.

Metabolism of Metronidazole and Antipyrine in the Rat

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The use of cocktails of probes provides ideal conditions for the study of co-regulation of their metabolic rates and more information regarding possible differential regulation of the involved enzymes. In the present study a cocktail of metronidazole (MZ) and antipyrine (AP) was assessed for the non-invasive study of hepatic drug metabolism in the rat.

As for AP the saliva-plasma ratio of MZ proved to be unity in 48 sample pairs from 6 anaesthetized rats (r=0.97). As for AP the clearance (CL) of MZ could be determined from one sample without loss of precision and accuracy compared with conventional determinations in 26 control, phenobarbital (PB) or ß-naphthoflavone (BNF) induced, or anaesthetized rats (r=0.99). In 6 rats administered MZ and AP, each alone and as a cocktail, the CL and fractional clearances of the two drugs could be determined from the same saliva sample and urine collected for 24 h without drug-drug interactions.

Three groups of 6 rats were treated with PB, BNF or nothing (control) prior to administration of MZ and AP as a cocktail. The total and fractional clearances were determined from one saliva sample collected after 4 h and urine collected for 24 h. PB increased the total and fractional CL of MZ to the acetic acid (MAA), hydroxy (HM) and glucuronide metabolite (GM) by 3-7 times. BNF increased the total CL of MZ and the formation rates of the oxidative metabolites, MAA and HM, by 5 times, but left the rate of glucuronidation unchanged. PB increased the total and fractional CL of AP to 3-hydroxymethylantipyrine (HMAP), norantipyrine (NORAP) and 4-hydroxyantipyrine (OHAP) by 4 times. BNF increased the total CL of AP by 4 times and the formation rates of NORAP and OHAP by 10 times but left the fractional CL to HMAP unchanged. In these 18 rats the formation rates of the three MZ metabolites correlated significantly with several of those of the AP metabolites (Table). However, the correlations between the metabolite formation rates and the CL of the other drug and between those of AP metabolites and the rate of glucuronidation of MZ were as high or higher than the correlations between the formation rate of the oxidative metabolites of the two drugs. Moreover, the latter correlations were generally related to the effects of the pretreatments and not apparent within each group of rats.

Correlations (r)	CL MZ	CL->MAA	CL->HM	CL->GM	
CL AP	0.81*	0.81*	0.85*	0.55*	
CL -> HMAP	0.63*	0.57*	0.19	0.75*	
CL -> NORAP	0.50*	0.38	0.64*	0.30	
CL -> OHAP	0.77*	0.70*	0.76*	0.69*	(* p<0.05)

It is concluded that a cocktail of metronidazole and antipyrine and non-invasive sampling are recommendable for the study of the differential metabolism of foreign compounds in rats *in vivo*.

300 µl Kapp.

Saliva sample + urine!

SC inj. pilocarpine 0.5 mg/rad (25°)

TRANSFORMATION OF N-ACETYLSULPHADIMIDINE IN HUMAN URINE.

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Sulphadimidine is a wide spread test drug used to determine an acetylation phenotype. The main sulphadimidine (SDM) metabolite is its N-acetylated derivative - N-acetylsulphadimidine (NASDM). Colorimetric methods used in clinical practice are indirect and, besides that, sensitive to different compounds having a NH_2 group rather than to SDM only.

The aim of our studies was to detect NASDM by direct HPLC method in urine of patients receiving SDM. The following effects were discovered during the chromatography of standard: i) chromatography of the standard NASDM solution reveals a single peak with a retention time (Rt) of 23 minutes, ii) after the standard solution is added to urine the peak of pure NASDM gradually disappears during one hour and a peak of transformed NASDM (Rt of 7.5 min.) simultaneously appears.

Chromatography of urine of the patients receiving SDM exhibited peaks corresponding to SDM itself (Rt - 6 min.) and transformed NASDM (Rt - 7.5 min.), whereas a peak of pure NASDM (Rt - 23 min.) has not been discovered. This is probably due to the fact that produced in liver NASDM in urine transformed another form whose chemical composition is not yet determined.

Thus, it has been shoun that the urine of patients receiving SDM contains transformed NASDM rather than pure one.

^{3}H NAVELBINE PULMONARY DISTRIBUTION STUDY IN RAT BY AUTOHISTORADIOGRAPHY. IN VITRO IN VIVO APPROACHES. QUANTIFICATION BY IMAGE ANALYSIS.

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Navelbine (vinorelbine DCI), a new anticancer agent, has been previously shown in animal experiments to highly concentrate in lung tissue. Consequently the aim of this work was to further investigate the pulmonary distribution of the drug under both in vitro and in vivo technique.

- In vitro, an isolated rat heart-lung preparation was used, perfused through pulmonary artery with 5 Mbq (0.4 mg) ^{3}H-NVB in Mac Coy's medium followed by repeated pulmonary washings.

- In vivo, ^{3}H-NVB was injected by I.V. route (1.5 $mg.kg^{-1}$ - 20 MBq) and animals were sacrificed after 30 and 120 minutes.

Autohistoradiographs (ILFORD K5 emulsion) were exposed for one month and developed with D19b.

Pulmonary uptake of radioactivity, as measured by liquid scintillation counting, was 0.2 MBq per gram of fresh tissue by both techniques.

Both approaches showed a uniform distribution of radioactivity over all alveolar and interstitial structures, however, in vitro exposure yields in the region of 5 times more silver grains than that of in vivo.

These findings are confirmed by examination of paraffin sections as well as semi-thin sections of araldite embedded tissue, where comparable uniform radioactivity levels in conjunctive tissue, smooth muscle, cytoplasm of epithelial cells, cytoplasm of endothelial cells and macrophages are seen. There is no particular binding to associated bronchial lymphoid formations although rare hotspots of labelling were seen in non-identified intra-capillar and pleural cells.

These quantitative observations are under confirmation, using a quantitative image analysis method.

PURIFICATION AND IDENTIFICATION OF A MAJOR METABOLITE OF 2833, A NEW HYPOLIPIDEMIC DRUG, IN HUMANS.

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In a previous study, we observed that most of the radio activity administered in 300 mg of 14C-2833 was excreted by the kidneys (unpublished results). Urine extracts were separated by thin layer chromatography, and 8 spots were observed by autoradiography. In order to get enough material to purify and identify the metabolites roughly scaled, a human volunteer was administered a 2400 mg dose. Urines were collected and added to a small amount of radioactive tracers, to control recovery. Enzymatic hydrolysis was performed just before extraction. Then High Performance Thin Layer Chromatography was associated with mass spectrometry to suggest metabolites structures.
A confirmation of the structure of the main metabolites precising the location of the oxydoreductions demonstrated by MS was obtained using NMR analysis. It confirmed the hypotheses suggested for the metabolic pathway of 2833 in humans.
A method for an isocratic HPLC determination was elaborated for 2833 and one of its main circulating metabolites in humans.

A STUDY OF THE CONTROL STRUCTURE OF N-ACETYL-4-AMINOPHENOL METABOLISM IN ISOLATED RAT LIVER CELLS

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The rate of N-acetyl-4-aminophenol (APAP) transport and metabolism (glucuronidation and sulphation) was measured in isolated rat liver cells. At 0.1mM APAP, control of glucuronidation is distributed equally between plasma membrane transport, glucuronidation and sulphation, with the latter exhibiting negative control on APAP glucuronidation. At higher APAP concentrations, flux control shifted from transport and sulphation to glucuronidation. The conversion of (0.1-5mM) APAP to its sulphate was controlled by factors associated with the uptake and/or metabolism of inorganic sulphate. This study elucidates the control structure of APAP metabolism in the liver over a range of APAP concentrations.

THE METABOLISM OF BENZO[A]PYRENE GENERATED BY CO-CULTURED HEPATOCYTES IS STRONGLY INFLUENCED, WHEN USING DIFFERENT RAT LIVER EPITHELIAL CELL LINES (RLEC) IN THE CO-CULTURING SYSTEM.

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Cultured hepatocytes are an intensively investigated in-vitro metabolizing system. The main limitation in using this in-vitro system to investigate the metabolism of drugs and other xenobiotics, is the decrease in drug metabolizing enzymes during the first hours of culturing.

Co-culturing freshly isolated hepatocytes with rat liver epithelial cells (RLEC) has been shown to stabilize various phase I and phase II enzymes during a period of 8 days, but to different extents (see accompanying presentation by D. Utesch et al.).

In the present study the metabolism of benzo[a]pyrene (BaP,80 µM) was used to characterize the metabolic capacity of hepatocytes (1 x 10^6/plate/24h) co-cultured with four different rat liver epithelial cell clones for 8 days. As the metabolic pattern of this polycyclic aromatic hydrocarbon is a result of the activity of monooxygenases, epoxide hydrolases, glucuronosyltransferases, sulfotransferases and glutathiontransferases, BaP metabolism can be used to define the metabolic status of cultured hepatocytes.

Four different epithelial cell clones (RLEC 1-5) were able to keep the parenchymal cells in good morphological status during an 8 day period of culturing. Furthermore the measurement of total BaP turnover (156 ± 11 pmol/10^6 hepatocytes/24h n=4) and the turnover to phase I metabolites (8,3 ± 1,1 pmol/10^6 hepatocytes/24h, n=4) showed no difference between the distinct epithelial cell lines used in the co-culture system.

In contrast to these results, the metabolic pattern of BaP generated by hepatocytes was strongly influenced when using cell lines (RLEC 1-5) as the co-culturing system.

	hepatocytes co-cultured with				suspended
ratio of metabolites	RLEC1	RLEC5	RLEC3	RLEC4	hepatocytes
BaP-9,10-dihydrodiol/ BaP-7,8-dihydrodiol	1,2	1,6 ± 0,1	0,6 ± 0,1	3,6	0,6 ± 0,1
BaP-9,10-dihydrodiol/ BaP-4,5-dihydrodiol	4,2	4,6 ± 1,1	1,4 ± 0,4	12,8	0,8 ± 0,1
total dihydrodiols/ total phenols	2,2	6,0 ± 3,8	1,1 ± 0,5	2,1	1,1 ± 0,2

Only hepatocytes co-cultured with one single epithelial cell line (RLEC3) were able to express the same metabolic pattern as freshly isolated hepatocytes.

These results demonstrate, that measuring the metabolic pattern of a complex metabolized substrate can be a more sensitive tool for characterizing the suitability of in-vitro systems in metabolism studies, than determining the activity of single enzymes,or total turnover rates.

METABOLISM OF BONNECOR® IN RATS.

Uwe Morgenroth, William Klemm, Volkmar Starke, Wilfried Zerjatke, Thomas Pallmer, Ulrich Joram and Alexander P. Rodionov.

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Metabolism of the novel antiarrhythmic drug Bonnecor® (3-carbethoxyamino-5-dimethylaminoacetyl-10,11-dibenz-[b,f] azepine hydrochloride) has been examined in rats. A general scheme of its metabolic pathway is presented. Possible influences of sex, route of administration, and chronic treatment have been checked. Metabolic data following administration of the main Bonnecor® metabolites were also obtained.

***In vitro* microsomal N-oxidation of 6-(pyridin-3-yl)-quinolin-2(1H)-one by different species.**

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The search for alternatives to the digitalis glycosides in the treatment of congestive cardiac failure has led to the introduction of several new agents. UK-57,400, 6-(pyridin-3-yl)-quinolin-2(1H)-one, is a prototype compound of a series which possesses positive inotropic and vasodilatory properties [1].

In dogs and rats, UK-57,400 undergoes rapid metabolism at the pyridyl nitrogen to the N-oxide which accounts for its short biological half-life. The extent of *in vitro* N-oxidation by liver microsomes has been examined in three species, rats, guinea-pigs and dogs. Due to the high substrate to product ratio, we have developed a specific HPLC assay to directly quantify the N-oxide formation in enzyme kinetic studies.UK-57,400, its N-oxide and the internal standard (8-methyl derivative of UK-57,400) are retained from the incubation mixture on a C18 Bond Elut® solid phase extraction column and eluted with methanol. The concentrated extracts are analysed by reverse phase HPLC on a Spherisorb® 5µm Phenyl column (12.5 x 0.46 cm), a quarternary mobile phase consisting of ammonium acetate buffer containing 2% TEMED (50mM, pH 3.6), methanol, acetonitrile and sodium acetate buffer containing 2% TEMED (50mM, pH 5) (90:15:18:60) at a flow rate of 1 ml/min, with UV detection at 254 nm. The calibration curves are linear for the range 1 to 10 nmoles/ml incubate. The coefficients of variation are below 5% at the tested concentrations of 1 nmole/ml and 10 nmoles/ml. Liver microsomal fractions from rats, guinea-pigs and dogs (n=3) metabolise UK-57,400 at the rate of 1.09 nmol/min/mg protein, 0.31 nmol/min/mg protein and 79.67 pmol/min/mg protein respectively. Further work is in progress to establish the involvement of cytochrome P-450 in this reaction, particularly the phenobarbitone, ethanol and pyridine inducible forms.

Reference

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3-GLUCURONIDATION/17α-ACYLATION OF ESTRADIOL-17α IN CATTLE : BIOSYNTHESIS OF A NEW CLASS OF COMPLEX CONJUGATES OF ESTROGENS.

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The recent study [1] on the biosynthesis of C-17 long chain fatty acid esters of estradiol-17B using bovine liver derived microsomes suggested a further hepatic metabolic step could occur and lead to glucuronide derivatives in position 3 of estrogen-17-fatty acyl esters. So, in the course of the reappraisal of estradiol-17B metabolism in veal calf, we have focussed our attention on the structure of conjugated estrogens excreted in bile.

After the hydrolysis of biliary estrogens with bovine glucuronidase and the solvent extraction of freed metabolites, about five percent of the total radioactivity were partitioned in a non-polar phase. These lipoïdal metabolites were cleaved only by alkali (KOH 5% in ethanol) but not by mild saponification with $NaHCO_3$. The main recovered radioactive metabolite was identified by HPLC on normal and reversed phase systems and by crystallization to constant specific activity. It was shown to be estradiol-17α which is the major metabolite of estradiol-17B in cattle (trace amounts of estradiol-17B were also detected). These non-polar metabolites further chromatographed on a Diol-column behaved the same as estradiol-17α-17 palmitate. Finally, these esters were separated as five major metabolites, the fatty acid moities being : arachidonate (15%), linoleate (27%), oleate (26%), palmitate (18%) and stearate (11%).

Altogether, these results are indicative of a mono-acylation of estradiol-17α on position 17 and a mono-glucuronidation on position 3. Consequently, the presence of this new class of metabolites of estradiol in bile confirmed the existence of an in vivo active hepatic estrogen-17-acylation and strongly suggests a further hepatic metabolism of estradiol-17α fatty acid esters as 3-glucuronides. So, estrogen-17-fatty acid esters that have been shown to be long acting estrogens due to their low elimination [2], can no longer be considered to represent a complete protective form towards degradative processes.

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COMPARATIVE IN VIVO METABOLIC STUDY OF TWO 2-AMINOPROPIOPHENONE DERIVATIVES

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Diethylpropion (2-diethylaminopropiophenone) and bupropion /2-(t-butylamino) -m-chloro-propiophenone/ are two 2-aminopropiophenones used as anorectic and antidepressant agent, respectively. While diethylpropion has been known for a longer time, bupropion is a newer derivative.

The aim of this work was to investigate in vitro metabolism of both derivatives, in order to compare in vitro metabolic pictures obtained between the two derivatives, as well as to compare and evaluate the results in respect to the known in vivo data.

The in vitro metabolism of these compounds was investigated using the fortified 9000 g supernatant and the washed microsomal fraction from homogenized rabbit liver. The incubations were carried out aerobically, at 37°C, for 30 to 60 minutes, with control experiments carried out concurently. The structures of the metabolic products formed were compared to standard compounds, obtained commercially or synthetized, and further identified using thin-layer chromatography (TLC), gas chromatography (GC), and both direct inlet and chemical ionization mass spectrometry (MS).

The results obtained showed extensive metabolism of both compounds, giving both dealkylated and reduced metabolic products, as well as deaminated ones. The main metabolites of diethylpropion obtained in vitro were formed by carbonyl reduction, alpha-C-oxidation followed by deamination, and N-deethylation followed by N-oxidation to give corresponding hydroxylamines and nitrones or amines. Bupropion is metabolized by direct N-oxidation, alpha-C-oxidation followed by deamination, carbonyl reduction, N-dealkylation followed by N-oxidation and, differently from diethylpropion, by oxidation of the bulky terc-butyl group, rather beta than alpha because of the absence of the hydrogen alpha to the nitrogen atom. The noticable differences in the metabolism of these compounds were based on structural differences, where bulky terc-butyl group of bupropion had the highest influence.

It is important to point out that very similar metabolic pictures were obtained with both compounds when the washed microsomal fraction was used instead of the 9000 g fraction.

THE FATE OF THE DIASTEREOMERIC GLUTATHIONE CONJUGATES OF α-BROMOISOVALERYLUREA IN BLOOD IN THE RAT IN VIVO AND IN THE PERFUSATE OF THE RECIRCULATING LIVER PERFUSION.

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Glutathione (GSH) conjugation is an important pathway in the elimination of xenobiotics. It is catalysed by several glutathione-S-transferase isozyme families, which show stereoselectivity towards chiral substrates such as α-bromoisovalerylurea (BIU): in vivo and in vitro work showed a more rapid conjugation and blood-elimination of (R)-BIU than of (S)-BIU (Polhuijs et al, 1989). The GSH conjugate of (R)-BIU was more rapidly eliminated in bile than that of (S)-BIU. Furthermore, the urinary excretion rate of the mercapturate from (R)-BIU was faster than that of (S)-BIU (te Koppele et al, 1986). This may reflect a more rapid GSH conjugation of (R)-BIU, but could also be due to a different handling of the GSH conjugates.

To determine the fate of the two diastereomeric GSH conjugates of BIU, (R) and (S)-IU-G, once they are present in blood, we injected the racemic mixture of the conjugates intravenously in the rat. Only very little (<5%) of the dose of the GSH conjugates was excreted in bile. Most (>70%) was further metabolized into the two diastereomeric mercapturates, which were excreted in urine with the same excretion rate, thus, showing no stereoselectivity. These results show that IU-G present in bile after administration of BIU, is formed almost exclusively in the hepatocyte. These results were confirmed in isolated, recirculating liver perfusions; the conjugates were not metabolised by the liver. The conjugates were also administered intravenously to rats with ligated kidneys; still very little IU-G was found in bile. In both in situ single pass and in recirculating liver perfusions it was found that the BIU enantiomers were excreted as (R) and (S)-IU-G in bile as well as in the perfusate.

In summary: (R) and (S)-IU-G formed in the hepatocyte, can be excreted both into bile and to the blood (perfusate); once the conjugates are present in blood (perfusate), they can be further metabolised in vivo into mercapturates and excreted as such in urine. Furthermore, a difference in urinary excretion rate of the mercapturates after administration of unconjugated BIU is most likely a result of a stereoselective GSH conjugation of BIU rather than of a stereoselective handling of the conjugates.

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Polhuijs, M. et al., J. Pharmacol. Exp. Ther. 249, 874-878, 1989.

LONG-TERM DISPOSITION OF SINGLE DOSES OF THE BISPHOSPHONATE PAMIDRONATE DISODIUM (CGP 23 339 A, AREDIA®) IN RATS

A. Probst and K. Schmid

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Pamidronate disodium (CGP 23 339 A, AREDIA®) is a potent inhibitor of bone resorption which acts by inhibiting osteoclast function. When given i.v. to rats its disposition is characterized by an extensive (up to 60% of the dose) and long lasting retention in the body, chiefly in the bones.

We have monitored the long-term disposition of pamidronate disodium given i.v. to rats by investigating its body-distribution (whole-body autoradiography) up to 6 months and its elimination from the body (renal excretion rate) up to about 17 months after intravenous administration of single doses of 1-10 mg/kg of ^{14}C-labeled compound.

Injected [^{14}C]pamidronate was rapidly cleared from circulation and taken up mainly by bones, liver, spleen, teeth and (calcified) tracheal cartilage. Radioactivity was eliminated from most soft tissues within 1 to 4 days. In liver and spleen it remained detectable for 1 and 3 months, respectively. In bones, trachea and teeth ^{14}C levels persisted up to the last observation time-point (6 months). In the bones, [^{14}C]pamidronate was gradually enclosed by newly formed bone tissue and was not redistributed within the bone itself to a measurable extent.

[^{14}C]Pamidronate not retained in the body was excreted predominantly via the kidneys within 4 days (20-30% of the dose). Thereafter, renal excretion of radioactivity continued at very low rates which declined multiexponentially. In the terminal phase the rates of renal ^{14}C excretion declined with a half-life in the order of 250-500 days. On the basis of the distribution studies it was concluded that during this terminal phase renal excretion reflected elimination of [^{14}C]pamidronate from the skeleton only.

Integration of the renal excretion rates showed that within the observation period of 17 months 50-60% of the [^{14}C]pamidronate disodium dose had been excreted with the urine. Assuming the contribution of biliary excretion to the total excretion of radioactivity to be negligible after day 4, it was deduced that 35-50% of the dose was still in the skeleton at the end of the observation period and that a large portion of the administered pamidronate would never be excreted. Comparison of the amount of radioactivity persisting in the tibia at the end of the experiment with that found in the tibia of different rats 1 day after dosing was in agreement with these figures.

KINETICS OF THE BISPHOSPHONATE PAMIDRONATE DISODIUM (CGP 23 339 A, AREDIA®) IN SELECTED TISSUES OF RATS AND MICE DURING AND AFTER REPEATED DOSING

A. Probst and K. Schmid

CIBA-GEIGY Ltd., Basel, Switzerland, Pharma Research & Development

Pamidronate disodium (CGP 23 339 A, AREDIA®) is a bisphosphonate used for the treatment of Paget's disease and tumor induced hypercalcaemia. We have investigated its distribution, accumulation and elimination in mice and rats during and up to eight months after daily i.v. treatment with 1 mg/kg of ^{14}C-labeled compound for 16 consecutive days.

In both species and at all time points investigated pamidronate was located predominantly in bones. The uptake of pamidronate in bone tissues varied among the bone samples investigated.

Upon repeated administration pamidronate accumulated in all biological samples investigated. In both species, the highest accumulation of pamidronate was found in bones, where the increase in concentration was almost directly proportional to the total dose administered.

The distribution and the accumulation of pamidronate in the body of rats and mice differed in that the uptake and the accumulation of the compound into soft tissues (particularly in the liver and the spleen) was distinctly more pronounced in mice than in rats, but in both cases was lower than in the skeleton.

After termination of the 16-day treatment pamidronate concentrations decreased very slowly in all investigated organs and tissues in both species. The slowest decline of pamidronate concentrations was found in the bones with terminal half-lives in the range of 150-250 days in rats and of 300-550 days in mice.

In the soft tissues the decline in pamidronate concentrations was more rapid than in the bones, with terminal half-lives which ranged from 80-150 days. In the plasma pamidronate concentrations declined approximately monoexponentially with a half-life of 25-50 days.

If the decline of concentration was corrected for the growth of the animals during the experiment, as exemplified for the tibia, the true elimination was estimated to proceed with terminal half-lives in the range of 400-450 days in rats and of 600-1200 days in mice.

FATTY ACID CONJUGATION OF A PYRETHROID INSECTICIDE

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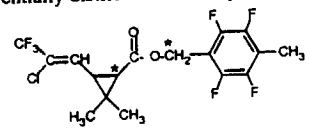
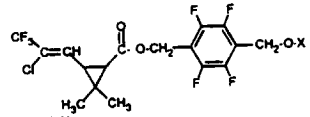
The tissue distribution of radioactivity in rats following single and repeated oral doses [^{14}C] labelled tefluthrin (I) demonstrated that the radioactivity was preferentially distributed into adipose tissue.

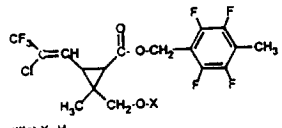


(I) * denotes [^{14}C]-atoms

However chromatographic analysis of these residues demonstrated that unchanged tefluthrin accounted for only *ca.* 50% of the radioactive residue. Detailed study of the unknown radioactive residues found in fat demonstrated that tefluthrin was first hydroxylated (IIa) and (IIIa) and then esterified with the fatty acids palmitic and oleic acid to produce the corresponding fatty acid ester metabolites (IIb,c) and (IIIb,c).



(IIa) X=H
(IIb) X=palmitate
(IIc) X=oleate



(IIIa) X=H
(IIIb) X=palmitate
(IIIc) X=oleate

These metabolites were unequivocally identified by mass spectrometry, the spectra being identical to that of chemically synthesised fatty acid esters.

THE PHARMACOKINETICS OF H 234/09 – A NEW CLASS III ANTIARRHYTHMIC DRUG – IN CONSCIOUS RAT.

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H 234/09 is a new class III antiarrhythmic agent, which is active both after oral and intravenous administration. The compound prolongs the duration of monophasic action potential in different animal species as well as in man. The pharmacokinetics of H 234/09 and the tissue distribution of tritium labelled compound was studied in male rats.

The pharmacokinetics were studied in starved male rats after single intravenous doses of 0.5 and 200 µmol/kg and single oral doses of 5 and 200 µmol/kg. Serial blood samples of 0.15 ml were drawn from the carotid artery during 24 hours after dosing. Unchanged H 234/09 was analyzed either by reversed phase HPLC separation with florescence detection, or by liquid scintillation counting.

H 234/09 was rapidly absorbed from the gastrointestinal tract and mean maximum concentration (C_{max}) was reached within 30 min post dose. The terminal half-lives of unchanged H 234/09 were 1 to 3 hours whereas the elimination of the total pool of metabolites was much slower. The bioavailability of the low dose was about 50 %. Renal and fecal excretion was of equal importance for the elimination of the dose.

The AUC increased disproportionately with increasing dose indicating non-linear pharmacokinetics of H 234/09 and saturation of the liver enzymes responsible for the elimination of the drug in the rat.

The radioactivity declined in parallel with that of blood and plasma in the majority of the 16 different tissues that was studied. The decline was rapid during the first 24 hours with 0.8 % of the given radioactive dose left in the tissues after the first day.

BIOTRANSFORMATION OF NITRENDIPINE IN THE DOG

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^{14}C-labelled Nitrendipine (3-ethyl 5-methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-[5-carboxy, 6-$^{14}C_2$]pyridine-3,5-dicarboxylate) was administered intraduodenally to a female Beagle dog (dose:5 mg/kg). Urine and bile (from bile fistula) were collected over a period of 24 hours.
The metabolite profiles in the excreta were established by HPLC (diode array detection, radioactivity monitoring). The unchanged drug was neither detected in the urine nor in the bile. Nitrendipine was extensively metabolized: 17 metabolites were isolated by LC and HPLC, resp., and identified by comparison with the reference compounds using HPLC with diode array detection, ^1H-NMR-spectroscopy, FAB-mass spectrometry and combined GC/MS after silylation. More than 85 % of the renally eliminated biotransformation products (approx. 33 % of dose) and about 83 % of the metabolites present in the bile (corresponding to 35 % of dose) have been identified.

The large number of metabolites was produced by some common biotransformation reactions:
- oxidative ester cleavage (also at the DHP state, not only at the pyridine state)
- hydroxylation of the methyl groups at 2- or 6-position (at the DHP and pyridine state)
- dehydrogenation of the 1,4-dihydropyridine system
- glucuronidation as phase II-reaction forming ester- and ether-type conjugates
- reduction of the aromatic nitro group (minor biotransformation reaction)

DISPOSITION OF THE ANALGESIC TRIAL DRUG CGP 29 030 IN RATS, DOGS, MARMOSETS AND BABOONS AND ANALGESIC ACTIVITY IN RATS AND MICE

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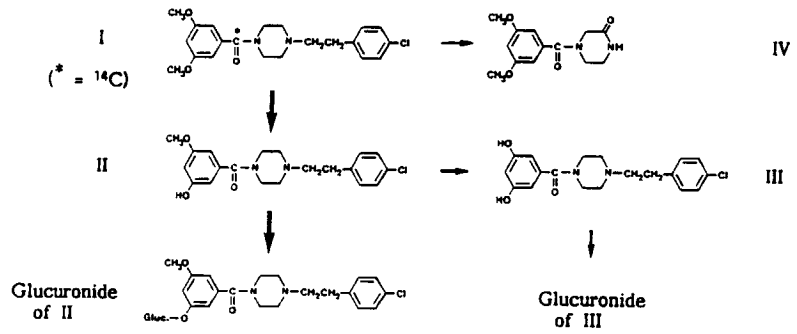
CIBA-GEIGY Limited, Basle, Switzerland, [1] Pharma Research and Development [2] Central Function Research

The absorption and disposition of the analgesic trial drug CGP 29 030 (I) have been investigated in rats, dogs, marmosets and baboons using ^{14}C- and non-labelled compound.

Orally administered CGP 29 030 was rapidly absorbed in all animal species investigated, the extent of absorption varied between 50 and 100%. Following 5 mg/kg i.v. and p.o. doses of [^{14}C]CGP 29 030 (as HCl salt) to rats, radioactivity was distributed widely throughout the body including the brain.

Excretion of radioactivity was almost complete within 4-7 days in all species; the major portion of the dose was excreted within the first 48 hours. In rats and dogs excretion proceeded mainly via the bile, while in marmosets and baboons the renal route predominated. CGP 29 030 was extensively metabolized; the pattern of metabolites in urine consisted of several compounds, virtually no unchanged CGP 29 030 was excreted in all species. The glucuronide of the desmethyl metabolite II was identified as the main metabolite in rat bile and in the urine of the other species. Further, the glucuronide of metabolite III was identified in urine of marmosets and the piperazinone metabolite IV in urine of rats. Metabolites II and III could be quantified by HPLC in plasma of marmosets. From these findings the main biotransformation pathways of CGP 29 030 are proposed as follows:



The analgesic activity of CGP 29 030 and its metabolites II, III and IV was tested in the PBQ-Writhing/Motility-test in mice and the Acetic Acid Writhing test in rats. All three metabolites were distinctly less potent than CGP 29 030 in both models.

DISPOSITION STUDIES WITH [^{125}I]-DEFIBROTIDE

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Defibrotide is an orally active antithrombotic and fibrinolytic agent composed of polydeoxyribonucleotides of molecular weight between 15,000 and 30,000 daltons. Previous studies have examined the plasma kinetics of biological activity associated with Defibrotide; no analytical methods of sufficient sensitivity to enable pharmacokinetic studies of Defibrotide in plasma have as yet been available. We have examined the blood kinetics of total radioactivity following oral and intravenous administration of [^{125}I]-labelled Defibrotide. The effect on blood kinetic profiles of radioactivity following pre-treatment of animals with potassium iodide (to prevent thryoid uptake) has also been examined. The time profile of total radioactivity in blood derived from administration of[^{125}I]-Defibrotide has been compared to that obtained following adminsitration of sodium [^{125}I]. Methods designed to distinguish between free radioactive iodide and [^{125}I]-labelled components derived from Defibrotide are described.

DENITROSATION OF A NOVEL NITROSOUREA, TAUROMUSTINE, BY GLUTATHIONE TRANSFERASE.

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The soluble or cytosolic glutathione transferases are a super family of multifunctional isoenzymes, which catalyze the conjugation of electrophilic substances with reduced glutathione. Due to protein sequence data and immunological properties, glutathione transferase can be divided into three distinct classes, named alpha, mu and pi. The different isoenzymes exhibit broad substrate specificities.

Nitrosoureas have been used into clinical oncology since late 1960s, and have been proved to be active cytostatic agents useful in a variety of malignancies. The antitumour effects are generally considered to their cytotoxicity via alkylation and cross-linking of DNA, a two-stage reaction which occurs when the nitrosoureas cleave spontaneously. Nitrosoureas can be inactivated by spontaneous degradation or denitrosation. Denitrosation is a reaction known to be catalyzed by the cytochrome P-450 monooxygenase system. Recently it has been demonstrated that denitrosation of N-nitroso compounds can be catalyzed by glutathione transferases as well. This reaction is suggested to be preferentially catalyzed by the mu class of transferase isoenzymes. The classes pi and alpha transferases demonstrate normally very weak catalytic activities towards N-nitroso compounds.

Tauromustine is a novel nitrosourea that can be enzymatically metabolized by two main routes in liver microsomes, namely demethylation and denitrosation. Here we describe the deactivation of tauromustine by denitrosation reaction catalyzed by glutathione transferases in liver cytosol from rat and mouse.

EFFECT OF VARIOUS DRUGS ON THE GLUCURONIDATION OF ZIDOVUDINE (AZIDOTHYMIDINE) IN HUMAN LIVER MICROSOMES

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Zidovudine (3'-azido-3'-deoxythymidine), formerly known as azidothymidine (AZT) is an analogue of the naturally occurring thymidine. It is at present the only drug with proven efficacy available for patients with AIDS or ARC. In man, the drug has a short half-life of approximately 1h, and is eliminated mainly by hepatic glucuronidation followed by rapid renal excretion of the glucuronide metabolite, GAZT (Cload, 1989). Interference of the glucuronidation of AZT by other drugs may therefore lead to enhancement of AZT effect or to increased toxicity of the drug. Such potential interactions may be of clinical importance as patients with HIV infection frequently require treatment with prolonged and multiple drug regimens. The present study describes an *in vitro* method which can be used for studying such interactions between AZT and other drugs which themselves undergo glucuronidation.

Microsomes were prepared using the classical differential centrifugation method from histologically normal human livers obtained from kidney transplant donors. Glucuronidation of AZT was carried out in a final volume of 0.2 ml containing 50 mM Tris-HCl (pH 7.5), 5mM $MgCl_2$, 5mM UDPGA, 4.5 to 9.5 mg microsomal protein/ml, and 0.25 to 10mM AZT. For studies with potential inhibitors, the concentration of AZT used was 2.5mM 1.5μCi/ml ^{3}H-AZT) and that of the inhibitors used ranged from 0.5 to 10mM. Incubations were carried out at 37°C for 1h and terminated with 0.1 ml acetonitrile. After centrifugation, 5 to 20 μl aliquots of the supernatants were assayed for AZT and GAZT with a reverse-phase C_{18} HPLC system (Good *et al.*, 1988). In cases where the inhibitor or its metabolite interfered with the uv detection of GAZT or AZT, radiochromatographic analysis was employed.

AZT glucuronidation followed Michaelis-Menten kinetics. The apparent K_m and V_{max} values (mean ± SD, n=5) were 2.60 ± 0.52 mM and 68.0 ± 23.4 nmol h^{-1} mg^{-1}, respectively, as determined from the Eadie-Hofstee plot. The V_{max} value obtained with the liver of a donor who had been on long-term phenytoin and phenobarbitone treatment (109.0 nmol h^{-1} mg^{-1}) was approximately twice that of other livers (66.1, 55.8, 54.4 and 54.7 nmol h^{-1} mg^{-1}). The effect of various drugs on AZT glucuronyltransferase activity is shown for the highest inhibitor concentration studied (10 mM) in the following table:

Drug	Percent control activity remaining
Indomethacin	1.9
Naproxen	5.0
Probenecid	19.2
Aspirin	35.3
Salicylic acid	40.5
Oxazepam	59.0
Paracetamol	96.6
Sulfanilamide	94.6

Further studies are in progress to characterise the inhibition observed with the drugs. Preliminary studies have indicated both naproxen and probenecid to be competitive inhibitors.

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BENZENE METABOLISM BY TWO PURIFIED, RECONSTITUTED RAT HEPATIC MIXED FUNCTION OXIDASE SYSTEMS

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Benzene is metabolized by the hepatic mixed function oxidase system. Post and Snyder (J. Tox. Env. Hlth. 11, 811, 1983) have demonstrated that rat liver microsomes contain at least two distinct mixed function oxidase activities, induced by phenobarbital or benzene, respectively, which can metabolize benzene. Benzene metabolism has now been further investigated using two purified rat hepatic MFO systems containing either cytochrome P450IIB1 (phenobarbital-induced) or cytochrome P450IIE1 (isoniazid-induced). Studies performed over a wide substrate concentration range (20 uM to 12 mM) indicate that P450IIB1 represents a relatively low affinity form of cytochrome P-450 with respect to benzene metabolism while P450IIE1 is substantially more efficient (10 to 20-fold) at low benzene concentrations (<320 uM). Cytochrome b_5 stimulated benzene metabolism by P450IIB1 approximately 2-fold, whereas metabolism by P450IIE1 was increased up to 6-fold. The metabolites observed in these studies were phenol (80%) and hydroquinone (20%).

When microsomal epoxide hydrolase was added to incubations containing the purified P450IIE1 MFO system, small quantities of benzene dihydrodiol (<5% of total metabolism) were detected. In the presence of a number of specific glutathione transferases, a putative glutathione conjugate (<15% of total metabolism) was also detected using the same mixed function oxidase system. These data suggest that benzene is initially oxidized via an epoxide intermediate which is preferentially rearranged, nonenzymatically, to its major metabolite, phenol which accounts for approximately 75% of the metabolites; hydroquinone, the dihydrodiol and the GSH conjugate account for 20%, 17% and 5% of the metabolism, respectively. The relatively small quantities of dihydrodiol and GSH conjugate formed may result from the low substrate concentration for the epoxide hydrolase or the GSH transferases because of the rapid rearrangement of benzene oxide. Alternatively, the epoxide may have a very low affinity for these enzymes. (Supported by ES 02931 and ES05022).

A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY AND PHARMACOKINETICS IN CONSCIOUS RATS OF ADD17014, A NOVEL TRIAZOLINE ANTICONVULSANT

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A sensitive and specific high performance liquid chromatographic (HPLC) method for the analysis of ADD17014 [1-(4-chlorophenyl)-5-(4-pyridyl)-Δ^2-1,2,3-triazoline], a novel anticonvulsant agent, in rat blood is described, together with preliminary pharmacokinetics following intravenous administration. ADD17014 is a representative member of a new class anticonvulsant compounds, the Δ^2-1,2,3-triazolines[1,2]. Traditional treatments for epilepsy rely on compounds that contain a dicarboximide or ureide function and these may contribute to the hypnotic and sedative effects experienced by many patients[2]. ADD17014 however lacks these structural moieties and hence could be predicticted to be less toxic[1,2]. Indeed ADD17014 is the most effective triazoline synthesised to date and affords seizure protection in a variety of animal models[3]. As part of the overall drug safety evaluation we proposed to study the metabolism and pharmacokinetics of ADD17014 in the rat. Following careful systematic trials , the following method was arrived at. ADD17014 and the internal standard (dipyridamole) were extracted into diethyl ether (5ml) from alkalinised blood (0.25ml blood plus 0.75ml pH 10.7 buffer), with extractability nearing 100% under these conditions. The assay is based on reversed phase HPLC (25 x 0.46cm spherisorb 5-ODS) using a mobile phase of methanol: acetonitrile: McIlvaine's citric acid-phosphate buffer (pH 8.0, 0.005M) (30:30:40, v/v/v) and UV detection at 290nm. Calibration curves were linear and reproducible (correlation coefficient > 0.999). Measurement of ADD17014 in rat blood (250μl sample size) was linear in the range 0 - 40 μg/ml and the coefficient of variation (COV) was less than 5%. The minimum detectable level was about 0.1μg/ml; however, a larger blood sample size (1 - 2ml) allowed measurement of levels as low as 10ng/ml, especially for estimation of drug levels in samples withdrawn at later time points (24hr). Preliminary pharmacokinetics in conscious rats at a dose of 20mg/kg show that the drug is cleared at a very fast rate. The β-elimination half-life was approximately 1 hour with a peak concentration of 8.8 ± 0.7μg/ml. The apparent volume of distribution was 1100ml and clearance was calculated to be 790ml/hr. More detailed pharmacokinetic studies in rats and other animal species are underway.

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EFFECT OF NON LINEARITY ON THE BIOAVAILABILITY OF ENANTIOMERS ADMINISTERED ALONE OR IN A RACEMATE.

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In a linear system, in which concentrations are proportional to the administered dose, the AUC, hence the bioavailability, is the same whether one enantiomer is administered alone or in a racemate. Under Michaelis-Menten kinetics, enantiomers (E_1 and E_2) in a racemate behave as competitive inhibitors and metabolic rate for one of the enantiomers is given by

$$v_1 = \frac{V_1 \; c_1}{K_1 \left(1 + \frac{c_2}{K_2}\right) + c_1}$$

in which the subscripts 1 and 2 refer to enantiomers E_1 and E_2, respectively. Under non linear conditions the bioavailability of enantiomer 1 will be higher when administered in a racemate as less enzyme will be available for its metabolism.
This is particularly true if E_2 is metabolized at a slower rate ; in that case concentrations of E_2 will be much greater than those of E_1 showing in vivo a greater apparent inhibition for E_2 than for E_1 as the dose increases. Simulations and actual data will illustrate this point.

METABOLISM OF CYCLOSPORIN BY HUMAN GASTROINTESTINAL MUCOSA *IN VITRO*

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Cyclosporin (CSA) is a potent immunosuppressive agent which is widely used to prevent the rejection of transplanted organs and increasingly to treat diseases of autoimmune origin. CSA is known to undergo extensive hepatic metabolism in man to mono- and dihydroxylated as well as N-demethylated products (Ptachcinski *et al.* 1986). Poor bioavailability of CSA has generally been attributed to a) poor absorption and b) hepatic metabolism, although a study by Gridelli *et al.* (1986) in dogs indicated some metabolism by the gastrointestinal mucosa. The aim of our study was to examine the potential of human gastrointestinal mucosa to metabolise CSA.

Histologically normal colon was obtained from six patients undergoing resection for localised tumours. The study was approved by the Mersey Regional Hospital Ethics Committee. Mucosal sheets were prepared and mounted between four pairs of perspex chambers as previously described (Rogers *et al.* 1987). Radiolabelled cyclosporin ([3H]-CSA; 17Ci. mmol^{-1}; 0.2μCi; 10μM) was added to each mucosal chamber. At intervals to 3h aliquots of buffer were removed from the mucosal chamber for determination of CSA and metabolites. Aliquots of mucosal fluid were subjected to extraction with diethyl ether and following evaporation to dryness were redissolved in mobile phase and analysed by radiometric hplc (Tjia *et al.*, 1989). Metabolites were tentatively identified according to the retention times of authentic standards (M17, M21). In one study gastric mucosa was used and a similar experimental protocol to that outlined above was followed.

The overall net transport of CSA from the mucosal chamber to the serosal chamber was low (1%). In the mucosal chamber at 3h (for colon), 77.6 ± 9.2% (mean ± S.D.) of drug was present as CSA, 9.9 ± 4.4% as M17 and 8.7 ± 4.2% as M21. Total metabolite production was variable (range 10.1-30.6%) and increased over the 3h period of incubation. Data obtained from the single gastric mucosal sample indicated metabolism of CSA to have taken place but the metabolites did not co-chromatograph with either M17 or M21.

The findings of this study indicate that CSA is metabolised in human gut mucosa *in vitro*. First pass metabolism in the gut may make an important contribution to the poor systemic bioavailability of CSA seen in many patients.

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CB 4261 : COMPARISON OF METABOLIC PROFILES (IN VITRO AND IN VIVO) STRUCTURE DETERMINATION OF METABOLITES FOLLOWING LC-MS ANALYSIS.

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The aim of this work was to compare the metabolic pattern obtained from in vitro (microsomes, hepatocytes, isolated perfused liver) and in vivo models (urine and bile), as well as to identify biliary metabolites in the rat (free and/or conjugated forms), using the LC-MS technique without sample pretreatment.

In the rat and in man, the radiochromatographic profiles from in vitro or in vivo models showed the same qualitative profile and only minor quantitative differences.

The comparison between rat biliary samples before and after enzymic hydrolysis showed that the unchanged drug and its metabolites were mainly excreted as glucuro-conjugates.

From LC-MS analysis of standards (either in their glucuro conjugates forms synthesized from microsomal fractions or in their free forms), structural determination of metabolites in rat biliary samples was carried out without any sample pretreatment.

IN VITRO COMPARISON OF THE METABOLIC PROFILES OF ALPIDEM WITH RAT AND HUMAN HEPATOCYTES IN PRIMARY CULTURE

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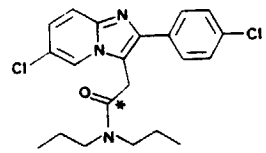
Alpidem, (ANANXYL^R), a new anxiolytic with selective binding to ω_1 receptors, is metabolized :
1- in the propyl side-chain by hydroxylation and N-dealkylation to active metabolites, which show a kinetic profile comparable to that of the parent molecule.
2- in the imidazo-pyridine ring to give glutathione conjugates.



* position of the ^{14}C in the labelled drug.

The aim of the study was to determine in vitro metabolism of alpidem using rat and human hepatocytes in primary culture and to compare the metabolic profiles to those obtained in vivo.
^{14}C-Alpidem was incubated with hepatocytes, then extracellular fluids were analyzed by HPLC with on-line radioactivity detection. Metabolites were identified by their retention behavior and by mass spectrometry (under various ionization conditions : EI-CI or FAB).

The profile of Phase I metabolites obtained in hepatocytes is similar to that obtained in vivo. Phase II reactions were also observed; particularly sulfur-containing peaks were identified. The pathway involving glutathion is more important in rats (35-44 %) than in humans (5-12 %).

Isolated hepatocytes from both rats and humans are a suitable model for predicting the in vivo metabolism of alpidem.

BIOTRANSFORMATION STUDIES IN RAT AND HUMAN LIVER SLICE CULTURES

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The use of liver slice cultures for biotransformation and toxicity studies is well described (Brendel et al., Tips 8:11, 1987). The goal of this study was to determine whether the biotransformation of pharmaceuticals in the organ slice cultures would predict the in vivo metabolites. The three pharmaceuticals included, the immunosuppressant Sandimmun® (Cyclosporin A, CsA), a dopaminergic agonist (CQA), and the antiemetic $5HT_3$-antagonist ICS.

Liver slices (200 ± 10 µm) were prepared freshly from rat (Sprague-Dawley 225g) and human tissue which was not transplanted, using a Krumdieck tissue slicer as described previously (In Vitro 22:707, 1986). The slices were maintained in a complete medium at 30°C with constant oxygenation (95/5; O_2/CO_2) and cultured individually on hanging baskets in 24 well tissue culture plates under gyratory shaking. Slices were preincubated for 2 h to allow recovery from slicing, the medium replaced and the slices exposed to the compounds for up to 24 h. DMSO was the vehicle used and accounted for less than 1% of the incubation volume. Slice viability was assessed on intracellular K^+ and Ca^{++} levels. The biotransformation of each compound was determined by the HPLC separation of parent and metabolites.

Cyclosporin A was metabolized to the known in vivo primary monohydroxylated (M1, M17) and the N-demethylated (M21) metabolites in the human and rat liver slice cultures. The secondary metabolites (M8, M13, and M18) resulting from further hydroxylation, N-demethylation and ring closure were produced in the human liver slices and to a lesser extent in the rat liver cultures. The extent of biotransformation, about 45% in the 18h human slices and 34% in the 24h rat slices reflects the clearance of the compound in vivo. The human interindividual variation of the three primary metabolites can be considerable, and has been shown to correlate with the amount of cytochrome P450III (Clin Pharmacol Ther 43:630, 1988). The rat kidney slices did not form any of the major metabolites, further supporting the evidence that the liver is the major site of CsA biotransformation.

CQA was well metabolized in the human liver slice culture, indicative of a high first pass effect. Metabolites of CQA (10 µM) were observed after 2h with 71% of the parent compound remaining. After 18h of culture 12% of CQA remained. In vivo CQA is also well metabolized in both rat and man, exhibiting a high first pass and low bioavailability.

ICS shows a low first pass in vivo and was metabolized slowly by the human liver slice cultures (10µM). Both major metabolites found in vivo, the 6-OH-ICS and 5-OH-ICS, were formed in these slice cultures. After 18h about 5% and 2% of ICS was metabolized to the 6-OH-ICS and 5-OH-ICS, respectively.

The biotransformation of the three compounds involves several P450 gene families and phase II conjugating enzymes. The formation of comparable metabolites in the organ slice cultures to the in vivo findings suggests the maintenance of the biotransformation enzymes in the slice system.

IX 207-887 GLUCURONIDATION IN RAT, DOG AND HUMAN LIVER MICROSOMES. INDUCTION OF GLUCURONYL AND GLUTHATHIONE TRANSFERASES.

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IX 207-887 is an antiinflammatory agent of a new type, the full effects of which develop more slowly than non-steroidal antiinflammatory drugs and corticosteroids. IX 207-887 is metabolized to the ketone 208-572 by cleavage of the enolic ether bond (minor pathway) and by conjugation of the carboxylic acid to the 1-O-acyl glucuronide (major pathway). To investigate species differences in drug metabolism at the enzymatic level, a study of the glucuronidation of IX 207-887 in rat, dog and human liver microsomal preparations was done. The effect of drug treatment on cytochrome P450 levels, glucuronyl and gluthathione transferase activities was assessed in rats and dogs.

The extent and site of IX glucuronidation was determined by HPLC analysis of Lubrol activated rat, dog and human liver microsomes incubated with [^{14}C] IX 207-887 (0.2 - 300 µM). The corresponding kinetics were evaluated using the Michaelis-Menten equation. Estimated Vmax values of IX 207-887 glucuronide formation in the rat, dog and human are 110, 390, and 190 pmols/min/mg protein, respectively. Treatment with IX 207-887 (150 and 200 mg/kg/day) for 11 weeks to rats and 8 weeks to dogs increased the Vmax of IX glucuronide formation 3.5 fold in the rats and 1.6 fold in the dogs.

Due to the chemical instability of acyl glucuronides and possible transacylation with the free sulphydryl group of cysteine residues the effect of IX 207-887 treatment on CDNB-GSH transferase activity was measured in rat and dog liver cytosol. Both male and female rats showed a dose dependent induction of CDNB-GSH transferase activity. At doses of 150 and 200 mg/kg/day male rats exhibited a 2.8 and 3.4 fold induction, while the females had a 1.6 and 2.2 fold induction. In the dog CDNB-GSH transferase activity was induced 1.5 fold in the male (200 mg/kg/day) and 1.9 fold in the female (150 mg/kg/day).

IX 207-887 treatment had a greater affect on the phase II conjugating enzymes which play more of a role in the metabolism of the compound. Cytochrome P450 levels were enhanced 2 fold in the rat but no change was detected in the dogs.

Identification of the induced forms of glucuronyl and GSH transferases is currently being studied. Furthermore other methods to monitor induction of the transferase reactions in humans are being investigated.

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P 4.66

COMPARISON BETWEEN VARIOUS MODELS FOR THE PRODUCTION OF GAMMA-GLUTAMYLTRANSFERASE.

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Gamma-glutamyltransferase (GGT E.C. 2.3.2.2.) plays an important role in clinical chemistry and pharmacology is a drug metabolizing enzyme able to transform some compounds to biologicaly active substances or prodrugs. For these reasons only the recent progress in the fields of cellular biology and/or genetic engineering can provide the adequate tools for production of the enzyme.

We have developed several models for the production of gamma-glutamyltransferase. Human hepatoma cell lines (Hep G2, PLC/PRF/5) were cultured under various conditions and/or on Cytodex microcarries, production of GGT was of 30 000 mU per liter of culture. The enzyme was also purified from these cells and its physicochemical properties were defined. The catalytic properties were found to be close to those of other human GGTs. For production in E. Coli, plasmids containing the cDNA sequences, coding for various parts of GGT were constructed. Depending whether or not the produced unglycosylated active enzyme contained its N-terminal hydrophobic region, different localizations (cytoplasmic or periplasmic) were obtained. Production of GGT was of 11000 mU per liter of culture.

Expression in S.cerevisial cells allowed the production of active enzyme localized in the cytoplasme. Finaly the full length DNA was expressed in V79 cells. High specific activities were obtained and the enzyme seems to be fully matured.

These models are very useful for the comparative study of the maturation steps of gamma-glutamyltransferase and are applicable to other drug metabolizing enzymes also.

P 4.67

SELECTION OF A CYTOSTATIC COMPOUND FOR HUMAN TRIALS BY STUDIES ON METABOLIC DISPOSITION OF THE DINALINE GROUP

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Dinaline (4-amino-N-(2'-aminophenyl)benzamide) and the chemical derivatives acetyldinaline (4-acetylamino-N-(2'-aminophenyl)benzamide) and methyldinaline (4-methylamino-N-(2'-aminophenyl)benzamide) are potential anticancer agents displaying activity against some tumors normally refractory to current drug therapy.

This presentation summarizes five other contributions at this workshop. The following metabolic aspects during comparative preclinical development are of primary importance: Is there a common major metabolite? Is the acetylation of the 4-aminogroup reversable? Are there species differences in metabolism? Does the acetylation step follow the genetic polymorphism of arylamine N-acetyl-transferase (NAT) enzyme activity in man?

Studies were conducted in mice, rats, dogs and monkeys. In-vitro biotransformation was investigated in human liver subcellular fractions. ^{14}C-labelled dinaline and acetyldinaline were available as well as specific HPLC assay methods for all three compounds.

Dinaline was rapidly absorbed in all species examined and extensively metabolized to acetyldinaline except in the dog. Following dinaline administration to rats, a fast and saturable conversion to acetyldinaline leading to nonlinear kinetics of dinaline in plasma was found. In monkeys, only low concentrations of dinaline were found up to 3 h postdose following dinaline administration.

The pharmacokinetics of acetyldinaline was dose-linear following acetyldinaline administration to rats. No dinaline was found in rats and monkeys following administration of acetyldinaline. This behavior suggests that N-acetylation is not a reversible process or that equilibrium is strongly on the side of the acetylation product.

After intragastric administration of radiolabelled dinaline and acetyldinaline to mice, rats, and monkeys, metabolic profiles were similar both intraspecies and interspecies for the two compounds. Acetylation seems to be slower in mice than in rats or monkeys. In all cases, acetyldinaline is the main compound excreted.

Acetyldinaline was the major compound detected in plasma and tumor tissue following equivalent single IG doses of dinaline, acetyldinaline, and methyldinaline. Tumor acetyldinaline concentration of approximately one-half the plasma concentrations were obtained following equivalent single IG doses. For all three dinaline compounds, acetyldinaline seems to be the in vivo active principle. The in vivo pharmacodynamic profile of all three compounds can be understood on the basis of the common active metabolite.

Dinaline-NAT activity was generally high among the six human liver cytosols, but showed no relationship to the N-acetylation of sulfamethazine. Acetyldinaline was not detectably deacetylated by human liver microsomes.

Thus, metabolic disposition studies contributed to the understanding of the similar spectrum of antitumor activity of the dinaline compounds and to the selection of the optimum substance for human trials.

METABOLIC DISPOSITION OF DINALINE IN RATS

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Dinaline (4-amino-N-(2'-aminophenyl)benzamide) shows marked antineoplastic activity against several types of malignant tumors. The aim of this study was to measure the absorption, biotransformation and elimination after oral administration of ^{14}C-dinaline.

^{14}C-dinaline (10 mg/kg) was administered IG in methocel suspension to male SIV-50 rats. Blood samples were obtained by retroorbital puncture up to 48 h, urine and feces were collected up to 144 h postdose. Bile was obtained from anesthetized bile duct cannulated rats over a period of 16 h.

Radioactivity in urine and bile was determined directly by liquid scintillation counting, blood and feces were combusted prior to counting. Plasma and urine were extracted with XAD 2 and eluted with methanol. The eluate was chromatographed on silica plates.

Mean maximum ^{14}C-concentrations of 9.1 ± 1.1 µg eq./mL (n=6) were reached 0.5 h postdose. Mean terminal radioactivity half-life was 5 h. Approximately 80% of radioactivity could be extracted from plasma. The dominant metabolite present was acetyldinaline (4-acetylamino-N-(2'-aminophenyl)benzamide). Trace amounts of dinaline were detected only in the 0.5 h plasma samples. Renal and fecal recoveries averaged 73.3 ± 4.3% and 19.4 ± 6.5% of dose, respectively. Only traces of residual radioactivity remained in liver and kidneys at 144 h postdose.

Mean biliary elimination (n=6) of total radioactivity over 16 h was 31.4 ± 8.5% with an average biliary half-life of approximately 5 h.

It is concluded that dinaline is rapidly absorbed from the gastrointestinal tract and is extensively metabolized mainly to acetyldinaline. The major route of radioactivity excretion is renal.

THE PHARMACOKINETICS OF UK-68,798, A NOVEL CLASS III ANTIARRHYTHMIC AGENT, IN MAN AND ANIMALS

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The pharmacokinetics of UK-68,798 [1-(4-methanesulphonamidophenoxy)-2-(N-(4-methanesulphonamide-phenethyl)-N-methylamino)ethane] have been studied in animals and man. Following intravenous doses, the volume of distribution in man was 3.9 L/kg and similar values were found in mouse, rat and dog, reflecting the similarity in plasma protein binding (60%). The plasma half-life was short in rodents (mouse 0.3h, rat 1.2h), due to high plasma clearance (mouse 235ml/min/kg, rat 37ml/min/kg). Longer half-life values were observed in dog and man (4.6h and 9.5h respectively) as a result of lower plasma clearance (10.2ml/min/kg and 4.7ml/min/kg respectively). Renal clearance of UK-68,798 was 80, 8.5, 3.0 and 2.5ml/min/kg in mouse, rat, dog and man. The reduction in renal clearance is largely commensurate with decreases in weight-normalised renal blood flow across the species. Hepatic clearance of the drug (plasma clearance-renal clearance) also declined across the species, being moderate in rodents but low in dog and man. This was reflected in the oral bioavailability of the compound which was 35% in rat, 66% in mouse, 72% in dog and 99% in man. The lower values in rat, mouse and dog reflect pre-systemic metabolism rather than incomplete absorption as similar recoveries of radioactivity in urine are observed in rat and dog after intravenous and oral doses of [^{14}C]-UK-68,798. In man, the complete bioavailability of UK-68,798 is in keeping with the low systemic clearance. In summary UK-68,798 has desirable pharmacokinetic properties in man for an antiarrhythmic compound having a moderate volume of distribution and half-life combined with complete bioavailability. In addition the equal balance in renal and hepatic elimination ensures that variation in renal or hepatic status will not cause dramatic changes in any kinetic parameter.

P 4.70

DIFFERENTIAL EFFECTS OF CALCIUM CHANNEL ANTAGONISTS ON CYCLOSPORIN METABOLISM BY HUMAN LIVER MICROSOMES.

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Cyclosporin (CSA) is a unique immunosuppressant used to prevent the rejection of transplanted organs and to treat diseases of autoimmune origin. CSA undergoes extensive hepatic metabolism in animals and man to mono- and dihydroxylated as well as N-demethylated products. The major primary oxidative metabolites are M1, M17 and M21. Further oxidation of M1 and M17 generate the dihydroxylated metabolites. Since the introduction of CSA a large number of clinically important interactions with other drugs has been reported. One area of particular interest at the present time is the use of Ca^{2+} channel antagonists in renal allograft recipients since diltiazem has been demonstrated to exert a beneficial effect on early graft function (Kohlaw *et al*, 1988). Ca^{2+} channel antagonists are agents of widely varying structure and it is important to ascertain how they affect CSA metabolism and hence CSA blood levels. We have previously demonstrated in human liver microsomal studies, that nicardipine is a potent inhibitor of the formation of both M17 and M21 whereas nifedipine and diltiazem are much less inhibitory (Tjia *et al*, 1989). The aim of the present work was to examine the effect of the Ca^{2+} channel antagonist isradipine on CSA metabolism in a comparative study with other Ca^{2+} channel blockers.

Histologically normal human livers (n=4) were obtained from renal transplant donors. Microsomal incubations (for 20 min) contained ^{3}H-CSA (5μM; 0.2μCi), NADPH (1mM), microsomal protein (1.5mg), $MgCl_2$ (5mM), EDTA (1mM), KCl (1mM), 1/15 M phosphate buffer (pH 7.4) to a final volume of 2.5ml. In some incubations, isradipine, diltiazem, nifedipine (5, 10, 20, 50μM) and nicardipine (1, 2, 5, 10μM) were added. CSA and metabolites were extracted into ether (6ml) and quantified by h.p.l.c. Separations were performed at 76° on a Partisil ODS-3 (25cm x 0.46 cm) column. The mobile phase used was acetonitrile: water (67:33) and the flow rate 1.5ml min^{-1}. Metabolites (M1, M17, M21) were identified on the basis of the retention time of the authentic compounds.

Nicardipine showed marked inhibition of CSA metabolism (IC_{50} for M17 production being 2μM). The other Ca^{2+} channel antagonists were very much less inhibitory. At a concentration of 50μM, (i.e. 10 X substrate concentration) isradipine produced 61% inhibition, nifedipine 47% inhibition and diltiazem 28% inhibition. Since the extent of inhibition seen in this type of study is dependent not only on the alleged inhibition concentration but also the substrate concentration it is more important to determine Ki values. We are currently doing this. However as a screening procedure the current study indicates that nicardipine has very much greater inhibitory effects on CSA metabolism than other Ca^{2+} channel antagonists.

Kohlaw K. *et al.* (1988). Transplant Proc 20, Suppl 2, 572-574.
Tjia J.F. *et al.* (1989). Br. J. Clin. Pharmac. 28, 362-365.

P 4.71

ω-OXIDATION OF ETHYLESTER GROUPS IN BENZODIAZEPINE ANTAGONISTS OF THE IMIDAZOBENZODIAZEPINE TYPE.

G. Vendt, D.E. Schwartz, G. Oesterhelt and G. Friedrich*

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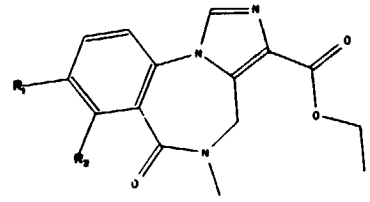
Drugs containing an ester function, especially with lower alcohols like ethanol, are usually rapidly metabolized by ester hydrolysis catalyzed by unspecific esterases ubiquitously present in mammalian organisms. Cleavage of the ester group, however, could also be achieved by oxidative attack of the α-carbon of the alcohol catalyzed by mixed function oxidases. Differentiation between the two pathways is difficult but it is normally assumed that ester hydrolysis is much faster than oxidative cleavage.
In the series of the benzodiazepine receptor antagonists of the imidazobenzodiazepine-3-carboxylate type



1) R_1 = F, R_2 = H: Flumazenil, ANEXATE^R

2) R_1 = H, R_2 = Cl

metabolites could be isolated from rat and dog urine that had been formed by oxidation of the methyl group of the ethyl ester side chain.
Though the free acid was the predominant metabolite in urine (70-90 % of urinary radioactivity), the products of ω-oxidation, the ethyleneglycol and the glycolic acid derivative, together accounted for up to 10-20 % of the urinary radioactivity in rats. These metabolites were less abundant in dog urine, and were not detected in human urine.
These results show that, at least in rats, oxidative attack at the ethyl group of an ester function can be competitive with hydrolytic cleavage of the ester. Hence formation of the free acid as main metabolite could be partly due to oxidative cleavage of the ester by attack at the α-carbon as well as to hydrolysis by esterases.

STEREOSELECTIVE BIOTRANSFORMATION OF PRAZIQUANTEL IN VITRO

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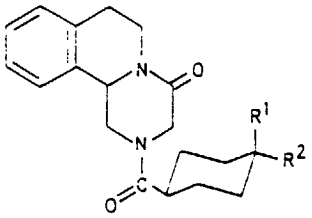
Praziquantel 1, the drug of choice for the treatment of schistosomiasis, is applied as a racemate (Biltricide®). The R(-)-enantiomer is therapeutically superior to the racemate, while the S(+)-enantiomer is almost ineffective. Previous studies in vivo showed that the drug undergoes stereoselective biotransformation in man and rat(1). Praziquantel is metabolized to different isomeric hydroxylated products.



	R^1	R^2
1	-H	-H
2	-OH	-H
3	-H	-OH

We wish to report studies on the in-vitro metabolism of the Praziquantel-enantiomers and the racemate. The drug-enantiomers were resolved by liquid chromatography on microcristalline cellulose triacetate as an optically active adsorbent. Praziquantel was incubated with liver microsomes from rat, rabbit, mouse and man. The analytical separation of the metabolites was achieved by reversed-phase HPLC using an acetonitrile/water gradient. R(-)-Praziquantel was metabolized mainly to the compounds 2 and 3 in all species , while in the case of S(+)-Praziquantel the pyrazinoisoquinoline moiety was hydroxylated. In contrast to the results of the in vivo studies, we found that in all investigated species metabolite 2 is produced in severalfold excess compared to metabolite 3.

(1) A.Kaup, doctoral thesis, Münster 1989.

METABOLISM OF REMOXIPRIDE IN DIFFERENT SPECIES

Marianne Widman, Lars B Nilsson, Britt Bryske and Jan Lundström

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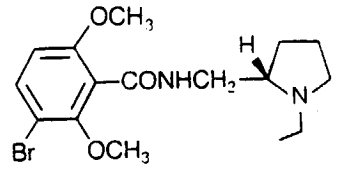
S-151 85 Södertälje, Sweden.

Remoxipride is a dopamine D_2-receptor antagonist which has been shown in clinical trials to have antipsychotic properties. The compound presently being evaluated for registration in several countries.



Remoxipride

(S)-3-Bromo-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamide

The metabolism of remoxipride has been studied in several species. In rodents (mouse, hamster, rat, rabbit), the predominant metabolic reactions occur at the aromatic ring i.e aromatic hydroxylation and O-demethylation. Resulting metabolites are excreted mainly as conjugates. However, in the dog as well as in man, metabolic reactions occur mainly at the pyrrolidine ring i.e oxidation at the carbon atoms alpha to the nitrogen. In dogs and in man most urinary metabolites are excreted in non-conjugated form.

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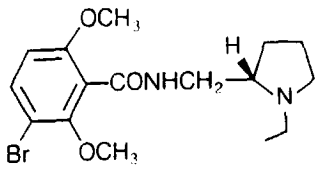

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METABOLISM OF CONTRACEPTIVE STEROIDS BY HUMAN BREAST CANCER CELLS IN CULTURE

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Human breast cancer cells lines are used extensively for the study of steroid hormone action. It is known that in both receptor positive and receptor negative cell lines there is considerable metabolism of the natural estrogens, estradiol (E_2) and estrone (E_1) with interconversion of the two steroids (17β-estradiol dehydrogenase enzyme activity) and formation of sulphate and glucuronide conjugates (Adams *et al.* 1988; Pasqualini *et al.*, 1989, Adams *et al.*, 1989). The aim of the present work was to see if the commonly used oral contraceptive steroids ethinyloestradiol (EE_2) and 3-keto desogestrel (3-ketoDg) were also metabolized in a human breast cancer cell line (MCF-7).

Cells were maintained in Dulbeccos Modified Eagles Medium (DMEM) containing foetal calf serum (FCS 5%), insulin (1 μg/ml) and epidermal growth factor (EGF; 5 ng/ml). On reaching confluence cells were transferred to DMEM containing charcoal-stripped FCS (5%) and insulin (1 μg/ml). 48 hours later radiolabelled steroid (^{3}H-E_1; ^{3}H-E_2; ^{3}H-EE_2; ^{3}H-Dg; 1nM; 0.2 μCi) was added and incubation was for 24 or 48 hours. Following incubation, the medium was removed and subjected to ether extraction followed by HPLC. Chromatographic separation of "Phase I" metabolism was performed on a μ-Bondapak C_{18} column with a mobile phase of methanol: ammonium dihydrogen phosphate (62:38), and of "Phase II" metabolites on a similar column with a mobile phase of methanol (initially 30% but increasing to 60% over 40 mins) and ammonium acetate. Radioactivity was determined by on-line radiometric detection. Metabolites were identified according to the retention times of authentic standards.

With E_2 as substrate, there was conversion to both E_1, E_2 3-sulphate and E_1 3-sulphate. With E_1 as substrate, there was extensive conversion to E_2 and also to E_1 3-sulphate and E_2 3-sulphate. These data confirmed the findings of others. With EE_2 as substrate there was no evidence of "Phase I" metabolites but marked conversion to the presumed 3-sulphate conjugate. With the progestogen 3-KetoDg there was evidence of both the 3α and 3β-hydroxy metabolites.

These studies have shown that two commonly used oral contraceptive steroids are metabolized in MCF-7 cells. Pasqualini *et al.* (1989) have recently reviewed the role of different estrogen sulphates in human breast carcinoma and concluded that they can play an important role in the biological response of estrogens. We await to see how this may apply to oral contraceptive steroids.

Adams E.F. *et al.* (1988). Int. J. Cancer 42, 119-122.
Adams J.B. *et al.* (1989). J. Steroid Biochem. 33, 1023-1025.
Pasqualini *et al.* (1989). J. Steroid Biochem. 34, 155-163.

P 4.76

The metabolism and pharmacokinetics of (-) 3 pyridyl pinacidil in Fischer-344 rats

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(-) 3 pyridyl pinacidil has been investigated as a potassium channel opening drug for use in the treatment of hypertension and asthma. In preclinical studies, the metabolism and pharmacokinetics of this compound have been investigated in Fischer-344 rats. At least one major metabolite has been isolated and characterised. After intravenous administration using a hydroxypropyl beta cyclodextrin complex, plasma concentrations were measured over a period of 8 hours. Estimates of pharmacokinetic parameters were made using NONLIN, and indicated that the compound was cleared rapidly in a monoexponential fashion. The volume of distribution was 0.87 l/kg, with a clearance of 2.3 l/h ($t_{1/2}$ = 0.26 h).

The rapid clearance of the compound mirrored its pharmacodynamic effects *in vivo* and measurements of the drug concentrations in lung tissue after i.v. dosing reflected those in plasma ($t_{1/2}$ = 0.25 h). This indicated that there was no selective uptake in that tissue. Information obtained in these studies will be used to design appropriate delivery techniques to extend the duration of action of the compound.

THE APPLICATION OF A METALLOPORPHYRIN AS CHEMICAL MODEL CATALYST IN THE INVESTIGATION OF THE METABOLISM OF THE ERGOT DERIVATIVE CQA 206-291

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Biopharmaceutical Department, Sandoz-Pharma Ltd., CH-4002 Basel, Switzerland

Metalloporphyrins have been widely used as chemical model systems for drug-metabolizing cytochrome-P450-dependent mono-oxygenases. The major use was made for investigations of the physico-chemical properties of the active site of the enzyme and for mechanistic studies of the oxygenation reaction. Up to now only few articles describe the application of such model sytems in the investigation of drug metabolism.

CQA 206-291, a CNS active ergot derivative, is metabolized *in vivo* in rat, dog and humans to a large number of metabolites. The identyfied phase I metabolites are formed mainly through N-dealkylation, hydroxylation and further oxidation.

The Mn-porphyrin Mn(TPP)Cl (TPP: meso-Tetraphenyl-porphyrin) has been applied for the oxidation of CQA 206-291, using Zn/HAc/O_2 as oxygen source. The products formed can be divided into three classes: 1) N-dealkylated products including formyl derivatives, 2) products derived from initial oxidation at the C-2/C-3 position of the indol ring and 3) products of aliphatic oxidation.
Therefore similar pathways exist in the oxidation with the model system compared to *in vivo* metabolism. Nevertheless a predominant *in vivo* pathway, the dealkylation at the sulfamid-moiety, was completly absent in the model oxidation. In contrast no metabolic reactions at the C-2 position of the indol was found in the major metabolites of rats. It seems that the reactivity of CQA 206-291 is partly shifted from the indole part to the sulfamid side chain by cytochrome P-450 compared to the model catalyst.